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Lysolecithinase of Cod Muscle[1]

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ABSTRACT

During the cold storage of cod fillets there is a gradual increase in the concentration of free fatty acids which are derived from the enzymatic hydrolysis of cod muscle phospholipid. One of the enzymes responsible, a lysophospholipase, was investigated in this study and its properties were found to be similar to those of mammalian lysophospholipases. Lysolecithin was hydrolysed to free fatty acids and glycerylphosphorylcholine; oleic acid strongly inhibited this reaction. The enzyme had maximal activity at pH 7.6 and it was deactivated by heating at 60°C. Muscle extracts kept their activity undiminished for several weeks at −9°C. The lysolecithinase activity of cod flesh was many times greater than its lecithinase activity. There were great, and yet unexplained, variations of activity among various cod.

INTRODUCTION

One of the changes occurring in fish fillets kept in frozen storage is a gradual increase in the concentration of free fatty acids. Fatty acids are mainly formed from the lecithin of fish muscle by enzymatic degradation (Lovern and Olley, 1962; Bligh, 1961; Lovern *et al.*, 1959) although phosphatidyl ethanolamine (cephalin) is also attacked. The breakdown of phospholipids stops after hydrolysis is 60–80% complete. This point is reached after about 15 weeks at −12°C or after 30 weeks at −18°C (Dyer and Fraser, 1959). Lipid hydrolysis occurs also during the processing of salted cod (Cardin *et al.*, 1958). The conversion of the fish muscle lipid would not be expected to have any direct influence on the taste and the quality of the fillets, but it is conceivable that the free fatty acids will induce changes of major significance, for instance, denaturation of proteins (Dyer and Dingle, 1961; Anderson and Steinberg, 1964). Alternatively, breakdown of the lipids (which are known to be constituents of the membranous structures of cells) might lead to a general breakdown of cellular structures with consequent release of hydrolytic enzymes. A study of the mechanism of lipid hydrolysis thus becomes a matter of practical importance.

One molecule of lecithin contains two fatty acids, one in the α- and the other in the β-position of glycerol, and it is likely that the breakdown proceeds stepwise through the action of two different enzymes (see Discussion). In preliminary experiments, it was noticed that cod muscle homogenates would hydrolyse lysolecithin at least ten times faster than lecithins. Some of the properties of the lysolecithinase of cod flesh will be reported.

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MATERIALS AND METHODS

Materials

Lysolecithin was prepared from egg lecithin by hydrolysis with snake venom (*Crotalus atrox*) (Hanahan *et al.*, 1960). The lysolecithin was passed through basic alumina in chloroform–methanol (1:1) to remove acidic by-products, which would interfere in assaying the enzyme.

Glycerylphosphorylcholine (GPC) was prepared by hydrolysis of egg lecithin with tetrabutylammonium hydroxide in diethyl ether (Brockerhoff and Yurkowski, unpublished data).

Enzyme Preparations

Atlantic cod (*Gadus morhua* L.) were used in all experiments. One part muscle and four parts water were homogenized for 3 min in a Waring blendor, centrifuged at 1300 × *g* for 1 hr, and the supernate was lyophilized and stored at –9°C until used.

Reaction System

The following conditions were used for the assays, unless otherwise specified. The reaction mixture (20 ml) contained 60 mg (0.11 mM) lysolecithin, 10 ml of 0.125*M* Tris buffer pH 7.6 (final molarity, 0.0625) and 148 mg of the lyophilized supernate. Incubations were performed at 25°C for 2 hr with agitation. Appropriate blanks were run with no substrate, which was then added after the reaction had been stopped.

Measurement of Enzyme Activity

Enzyme activity was measured by the amount of fatty acid formed after a certain period of incubation by the following procedure: the fatty acids were extracted according to the (slightly modified) method of van der Vies (1955). The enzymic reaction was terminated by the addition of 20 ml of 5% phosphoric acid in 25% sodium chloride solution followed by 80 ml of ethanol and 50 ml of hexane. The mixture was then shaken for 1.5 min. After filtration of the hexane layer through paper, an aliquot was reduced to dryness and dissolved in 15 ml of a neutralized solution of ethanol–benzene–water (20:10:1) containing 3 drops of phenol red indicator. The titration was carried out under nitrogen with 0.01*N* ethanolic sodium hydroxide. The specific activity is defined as mμM/hr/mg of enzyme preparation (dry weight of homogenate, supernate, or residue).

Determination of L-α-glycerylphosphorylcholine (GPC)

After incubation of lysolecithin, under conditions as given above, 10 ml of 5% bovine albumin were added followed by 5 ml of 49% trichloroacetic acid. Five minutes later the sample and blank were centrifuged for 10 min. The supernate was extracted with diethyl ether three times to remove the

trichloroacetic acid and the aqueous layer was passed through a mixed-bed, Amberlite MB-3 resin. The eluate was reduced to dryness and diluted with water to the required concentration. The sample and blank along with standard solutions of choline, phosphoric acid, glycerolphosphate, phosphorylcholine, and GPC were chromatographed on paper by the ascending technique with phenol saturated with water–acetic acid–ethanol (100:10:12) (Dawson, 1960). GPC was detected with Dragendorff reagent (Mangold, 1961), with a phosphomolybdate spray (Dawson, 1960), and with silver nitrate (Trevalyan *et al.*, 1950). The spots from sample and blank corresponding to GPC were then cut out and analysed for phosphorus.

RESULTS

The lysolecithinase activity of muscle from different cod was variable. Therefore, it was decided to survey the activity of some frozen stored cod fillets, fillets from freshly caught cod, and from fish starved for 2 weeks, in the hope of finding a source of reasonably high activity. Table I gives the description and the lysolecithinase activity of the fish surveyed. The weights of the livers, expressed as per cent of body weight, give an indication of the nutritional state of the fish. All samples were taken from the region of the lateral line below the first ray of the second dorsal fin. The range of specific activity of various fish was 0.34–6.73 mμM/hr/mg, about a 20-fold variation. The fish that had been freshly caught tended to have higher specific activities than those that had been starved and the fish (No. 15) with the highest specific activity apparently had not fully recuperated from spawning. Otherwise, the data obtained so far do not furnish an explanation of the differences in enzymic activities. The lyophilized cod muscle supernate used in the following experiments was prepared from the combined fillets of fish 15, 16, and 20. Total and specific activity of lysolecithinase in the homogenate, supernate, and residue of cod muscle were compared (Table II). The supernate contained 56–60% of the total activity and the specific activity was three times greater than that of the homogenate. There was no loss of activity on partial purification.

Characterization of Lysolecithinase

The change of activity of lysolecithinase with time when the starting substrate concentration is near its optimum is shown in Fig. 1. The rate of formation of fatty acids was linear for about 2 hr and then began to decrease. This may be due to decrease in substrate concentration and inhibition by the fatty acids formed. The change of specific activity with temperature (Fig. 2) showed that the temperature optimum for cod muscle lysolecithinase is approximately 40–42°C. Below 29°C, 1-hr incubations yielded lower specific activities than 4-hr incubations but from 29 to 75°C the specific activity was always greater after the shorter time. It would thus appear that at the temperatures below 29°C there is a lag period for the initiation of hydrolysis.

TABLE I. Description and lysolecithinase activity of individual cod. (Samples to be assayed were taken from the region of the lateral line below the first ray of the second dorsal fin. The incubation mixture contained 2 g of homogenized muscle, 0.09 mM (50 mg) of lysolecithin, 0.08 mM calcium chloride, 0.5 mM Tris buffer of pH 8.4. Water was added to make 20 ml, and the mixture was incubated at 25°C for 5 hr. Fish A–E had been frozen for 110 days before analysis. Fish 7–20 were assayed when freshly caught. Fish 1–6 had been kept for 2 weeks at 7°C with no feeding. Fish 15 appeared to be recuperating from spawning.)

Fish number	Length (*cm*)	Weight (*g*)	Sex	Gonads weight (*g*)	Liver weight (*g*)	Percentage liver of total weight	Specific activity (*μM/hr/g fresh muscle*)	Specific activity (*mμM/hr/mg solids*[a])
A	73	3319	M	–	29	0.9	0.53	2.7
B	66	2835	M	–	80	2.8	0.33	1.7
C	62	2013	F	–	26	1.3	1.53	7.7
D	64	2183	M	16	42	1.9	0.83	4.2
E	63	2013	F	8	38	1.9	0.36	1.8
1	49	977	M	3.5	33	3.4	0.69	3.5
2	47	826	F	4.5	27	3.3	0.46	2.3
3	49	1080	F	6.5	38	2.6	0.72	3.6
4	51	1630	F	6	45	2.8	1.06	5.3
5	52	2415	F	8	39	1.6	0.55	2.8
6	85	4540	F	22	256	5.8	0.34	1.7
7	64	1905	F	9	69	3.6	1.95	10.0
8	56	1765	M	6	60	3.4	0.77	3.9
9	55	1563	F	–	45	2.9	0.39	2.0
10	59	1534	F	6	61	4.0	0.47	2.4
11	61	1820	F	9	48	2.6	2.42	12.1
12	50	1080	F	–	46	4.3	0.66	3.3
13	54	1534	F	7	47	3.1	0.63	3.2
14	43	855	F	3.5	26	3.0	0.65	3.3
15	77	3590	F	39	139	3.9	6.73	33.8
16	69	3015	M	sm[b]	150	5.0	4.28	21.4
17	71	3490	F	15	168	4.8	1.82	9.1
18	65	2420	F	11	56	2.3	1.13	5.7
19	63	1760	M	sm[b]	78	4.3	1.06	5.3
20	58	1930	F	7	53	2.8	4.14	20.7

[a]Assumed that water content of fresh muscle is 80%.
[b]Small, less than 2 g.

TABLE II. Lysolecithinase activity in cod muscle homogenate, supernate, and residue. (Incubation mixtures of 20 ml contained 10 g homogenate or equivalent amounts of supernate and residue, 0.093 mmole of lysolecithin and 0.5 mmole of Tris buffer of pH 8.4, and were incubated at 25°C for 5 hr.)

Fraction	Total solids in fraction (*mg*)	Total activity (*mμmoles/hr*)	Specific activity (*mμmoles/hr/mg solids*)
Fish 21			
Homogenate	400	1020	2.6
Supernate	84	620	7.4
Residue	316	420	1.1
Fish 22			
Homogenate	400	10812	27
Supernate	71	6034	85
Residue	329	4600	14

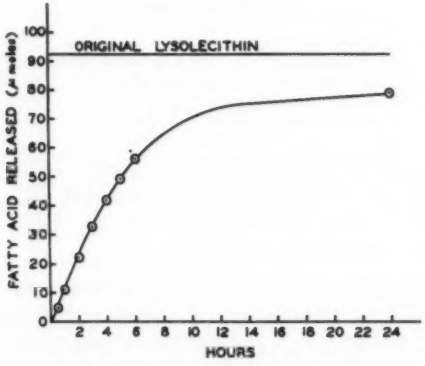

FIG. 1. Time course of hydrolysis of lysolecithin. A reaction mixture of 200 ml was prepared as described under Methods except that 0.0925 mmole of lysolecithin and 0.5 mmole of Tris buffer of pH 8.4 per 20 ml of reaction mixture were used. Aliquots of 20 ml were taken at the various intervals.

The influence of substrate concentration on the specific activity of lysolecithinase at the optimum temperature is shown in Fig. 3. The optimum substrate concentration was about $1.3 \times 10^{-4} M$ of lecithin for our reaction conditions. The Michaelis constant (K_M) for lysolecithinase under these conditions is 2.7×10^{-5}.

The effect of hydrogen ion concentration (pH) on the specific activity at 25°C and at near optimum lysolecithin concentration is given in Fig. 4. The

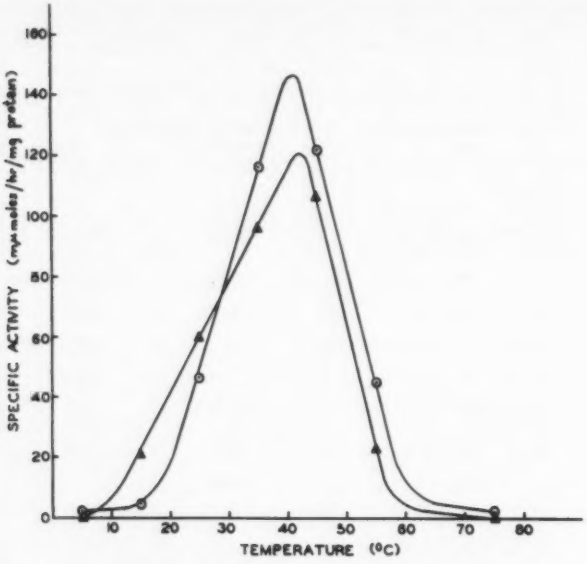

FIG. 2. The effect of temperature on the activity of cod muscle lysolecithinase. The reaction mixture was as described under Methods except that Tris buffer of pH 8.0 was used. ○: 1-hr incubation; △: 4-hr incubation.

pH optimum for lysolecithinase under these conditions is 7.6 and at the physiological pH of cod muscle (6.5–7.0) there is about 70% optimal enzymatic activity. Incubation in Tris buffer gives slightly higher activity than does NaH_2PO_4–Na_2HPO_4 buffer of the same pH.

The heat deactivation of lysolecithinase is shown in Table III. At 50°C preincubation temperature, there was a slight activation of lysolecithinase, while at 60 and 75°C there was 61 and 100% inhibition of activity, respectively.

The effect of various inhibitors on the activity of cod muscle lysolecithinase is given in Table IV. Mercuric chloride and *p*-chloromercuribenzoate inhibited the activity of lysolecithinase by 48% and calcium chloride by 20%. Phenylmethylsulfonylfluoride (PMSF), an esterase inhibitor (Fahrney and Gold, 1963), inhibited the enzyme by 23%, while L-1-tosylamido-2-phenylethylchloromethylketone (PTCK), which binds the histidine on the active center of chymotrypsin (Schoellmann and Shaw, 1963), inhibited by 41–57%. Isopropanol and methanol used in aiding the solution of PTCK and PMSF inhibited the enzyme by 4 and 13%, respectively. Diethyl ether was also inhibitory by 16%. Lecithin had a slight inhibitory effect, and oleic acid inhibited the activity of lysolecithinase from 70 to 80%. Iodoacetic acid, potassium cyanide, sodium fluoride, and EDTA have little or no inhibitory effect.

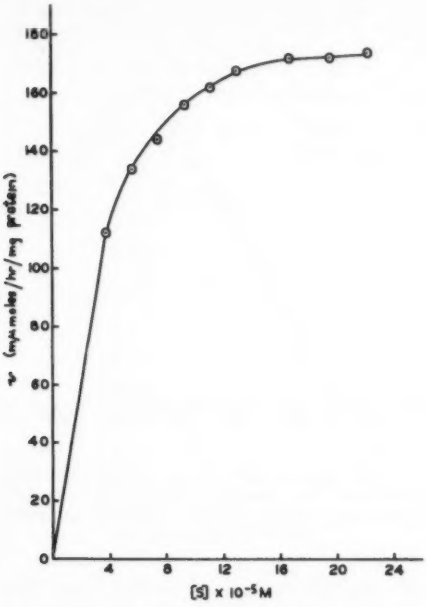

FIG. 3. The effect of substrate concentration on the activity of cod muscle lysolecithinase. The reaction mixtures were prepared as described under Methods except the pH of the Tris buffer was 8.0 and the incubation temperature was 40°C. Various concentrations of lysolecithin were used. The velocity (v) of lysolecithinase reaction against the substrate concentration, [S], was plotted.

The identity and amount of glycerylphosphorylcholine (GPC) were determined and compared to the amount of fatty acid formed in the breakdown of lysolecithin by the lysolecithinase preparation. A reaction mixture of double volume was run as described under Materials and Methods. After incubation one-half was used to determine the fatty acids; the other half was used for the determination of amount and identity of GPC. A blank was run similarly but lysolecithin was added after the reaction has been stopped. According to a phosphorus determination (see Materials and Methods) 27 μmoles of GPC were recovered against 25 μmoles of fatty acid. This shows that the reaction product was GPC, which was not further degraded.

DISCUSSION

The results of this investigation show that the lysolecithinase of cod muscle is similar in its properties to the lysolecithinases of mammalian tissues. Like these, it occurs in much higher activities than the accompanying lecithinase (Dawson, 1956; Epstein and Shapiro, 1959; Marples and Thompson,

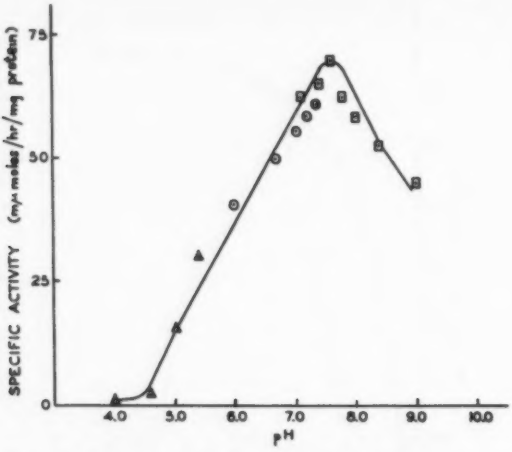

FIG. 4. The effect of pH on the activity of cod muscle lysolecithinase. The reaction mixture was prepared as described under Methods. △: acetate buffer; ○: phosphate buffer; □: Tris buffer.

1960). The biological significance is obvious; lysophospholipids have the faculty of disrupting cells, therefore they are not allowed to accumulate. Accordingly, only traces of lysolecithin are present in cod muscle, and the concentration does not increase during cold storage (Lovern *et al.*, 1959; Bligh, 1961; Olley *et al.*, 1962; Brockerhoff and Hoyle, unpublished data).

Lysolecithinases are not inhibited by EDTA and thus hydrolysis is independent of bivalent cations, such as calcium. In agreement with this result, it was found in this laboratory (Brockerhoff and Hoyle, unpublished data) that treatment of cod fillets, or minced cod, with EDTA will not prevent the hydrolysis of phospholipids. Likewise, polyphosphate treatment, which would

TABLE III. Heat-inactivation of cod muscle lysolecithinase. (The conditions of incubation were as described under Methods, except that the reaction mixture was preincubated at desired temperature for 10 min and cooled to 25°C prior to addition of the substrate.)

Preincubation temperature (0°C)	Specific activity (mμmoles/hr/mg)	Per cent inhibition
25	129	–
50	142	−11[a]
60	51	61
75	0.3	100

[a]Activation.

TABLE IV. The effect of inhibitors on the activity of cod muscle lysolecithinase. Incubation conditions are given under Methods. (*p*-CMB = *p*-chloromercuribenzoate; PMSF = phenylmethylsulfonylfluoride; PTCK = L-1-tosylamido-2-phenylethylchloromethylketone; EDTA = ethylenediaminetetraacetic acid (disodium salt).)

Inhibitor	Concentration mM	Specific activity (*mμmoles/hr/mg protein*)	Per cent inhibition
Mercuric chloride	0.02	40.0 (76)[a]	48
p-CMB	0.02	39.1 (76)	49
Iodoacetic acid	0.02	85.4 (84)	0
Potassium cyanide	0.02	83.5 (84)	0
Sodium fluoride	0.02	76.2 (76.2)	0
Sodium fluoride	0.40	79.5 (84.0)	6
EDTA (disodium)	0.02	72.7 (76.2)	5
Calcium chloride	0.81	67.1 (80.8)	17
PMSF (1 ml isopropanol)	0.02	61.5 (79.3)	23
PTCK (1 ml isopropanol)[b]	0.02	46.5 (79.3)	41
PTCK (1 ml methanol)[b]	0.04	30.0 (80.0)	57
Isopropanol	1 ml	77.0 (80.0)	4
Methanol	1 ml	69.0 (80.0)	13
Diethyl ether	2 ml	70.7 (84.0)	16
Glycerol	2.75	83.8 (84.0)	0
Lecithin	0.075	71.2 (79.3)	10
Lecithin	0.125	63.1 (80.8)	22
Oleic acid	0.09	31.0 (80.0)	71
Oleic acid	0.18	14.0 (79.3)	82

[a]Controls in parentheses.
[b]PTCK was not completely soluble in the incubation mixture.

also be expected to bind calcium, is without effect on lipid hydrolysis (Dyer *et al.*, 1964). Mercury and *p*-chloromercuribenzoate, which react with cysteine, cause partial inhibition; iodoacetate, which also blocks cysteine, does not. Similar results were obtained for mammalian lysolecithinase (Dawson, 1956); perhaps mercury compounds cause a nonspecific precipitation of the protein. Compounds blocking serine (PMSF, Table IV) and histidine (PTCK, Table IV) inhibit the enzyme; the two amino acids are probably constituents of the "active centre," as they are in most hydrolylic enzymes.

The most interesting result among the inhibition studies is the strong inhibitory effect of oleic acid. This probably explains why the hydrolysis of phospholipids in frozen cod fillets slows down and then ceases after 60–80% have been hydrolysed.

There remains the question whether the phospholipids are broken down by one enzyme or by two. Present consensus favours the existence of two enzymes, phospholipase and lysophospholipase, in animal tissues. In preliminary experiments on the lecithinase of cod muscle we found that while activity could be demonstrated in fresh extracts, none was left after several weeks storage at −9°C whereas the lysolecithinase activity was virtually undiminished. Similar findings were reported for the enzymes of rabbit polymorphonuclear leukocytes (Elsbach and Rizack, 1963). This supports the assumption that there are two enzymes of different stability present. It is possible that the number of enzymes is even greater, since lysophospholipids could occur as positional isomers (α- and β-acyl compounds) and phospholipids could be hydrolyzed in either α- or β-position by different enzymes. Again, there might exist an isomerase which converts one lysophospholipid into its isomer.

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Chalkiness in Halibut in Relation to Muscle pH and Protein Denaturation[1]

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ABSTRACT

Chalkiness in halibut, a condition in which the flesh is dull, white, opaque, soft and flabby in contrast to the normal shiny, translucent, and firm appearance, has been found to be a post-mortem development; the flesh of normal and potentially chalky fish being indistinguishable in appearance when they are caught. The change to the chalky condition was shown to be dependent on the pH of the muscle, taking place only if it fell to about 6.0 or lower, and to be accompanied by a marked decrease in extractability of both myofibrillar and sarcoplasmic protein. Denaturation of these proteins is probably the direct cause of chalkiness. Possible reasons for the rather wide range of muscle pH found in halibut taken in the British Columbia fishery are discussed.

INTRODUCTION

For many years chalky halibut have been of concern to the fishing industry of the Pacific Northwest, for, although chalky fish are apparently equal or superior to normal fish nutritionally (Bailey, 1951), the appearance of their flesh leads to frequent rejection by consumers. In contrast to the shiny, semi-translucence of normal raw flesh, chalky flesh is dull and opaque, and has been said to have a cooked appearance (Patashnik and Groninger, 1964). In addition, chalky halibut is softer and flabbier than normal (Bailey, 1950), and the myotomes of chalky flesh tend to separate from each other more readily than do those of normal flesh.

An examination of the literature and an inquiry among people connected with the industry revealed that there was no known cause of chalkiness and that it was not known whether the condition was present in the flesh when the fish were caught or if it developed post-mortem. Bailey (1950, 1951) found that the protein and oil content was higher and that of water lower in chalky flesh. However, it did not appear probable that these quantitative differences alone could account for the remarkable difference in appearance, and Patashnik and Groninger (1964) have reported an analysis of chalky halibut in which moisture, protein, and oil content were found to be within the normal range. Bailey (1950) reported that his analytical results "and other observations" provided no evidence to indicate that chalkiness was a sign of a diseased condition of the fish.

The present investigation was undertaken in an attempt to ascertain the cause of chalkiness in halibut.

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MATERIALS AND METHODS

Halibut were caught by dragging in drags of 1–3 hr duration. On board, examination for chalkiness was made by observing the surface of a cut made across a wide portion of the body of the fish. On shore, a similar examination was made at the time of fletching. Fish were classified as being normal, moderately chalky, or very chalky. Samples of muscle were taken at the time of catching the fish and again at the time of landing. In some instances, additional samples were taken at periods of up to 7 hr after catching before the fish were iced. All samples were taken from a position near the middle of the underside of the fish and were placed at once in closed polyethylene bags and frozen on dry ice in an insulated box for transportation to the laboratory, where they were stored at −30°C until analyses could be carried out. After being caught, examined, and sampled, fish were eviscerated and iced, in accordance with the usual commercial practice, for transportation to shore.

A number of samples of fish caught by longline was obtained after being landed in ice, 2–3 weeks following catching.

For measurement of pH, 2 g of muscle (without prior thawing if frozen, unless indicated otherwise) were homogenized in 20 ml of 0.005*M* sodium iodoacetate solution (Partmann, 1960). Measurements were made with a Beckman model H pH meter. Lactic acid was measured by the method of Barker and Summerson (1941), extracts for analysis being prepared by homogenization of frozen muscle samples in ice-cold 10% trichloracetic acid solution. Protein nitrogen was determined by the method of Torten and Whitaker (1964). In our experience there is a loss of about 5% during the 10-min heating period used in the extraction when halibut muscle protein is subjected to this procedure. The results reported below have not been corrected for this loss. Protein soluble in high-ionic-strength salt solution (myofibrillar and sarcoplasmic protein together) was extracted by the method of Dyer *et al.* (1950), as slightly modified by Tomlinson and Geiger (1963), and protein soluble at low ionic strength (chiefly sarcoplasmic protein) (Connell, 1962) in 0.025*M* KCl, 0.039*M* borate, pH 7.1 (Perry, 1953) as described previously (Tomlinson and Geiger, 1963). The dry weight of muscle was determined by drying 10-g samples to constant weight at 105°C.

In order to observe changes in the translucence of muscle during and after thawing, slices about 2 mm thick were cut from frozen samples, placed on a black plastic background and protected from drying by means of a clear plastic cover. They were then allowed to thaw in air at 20°C. For measurement of changes in protein solubility during thawing and after, frozen samples (2.5 g) were cut into small pieces, wrapped in aluminum foil, and allowed to stand in air at 20°C for the length of time desired. Such samples were thawed within 8 min.

RESULTS

Visual Observations

In preliminary work during 1963 a small number of fish was examined. None was found to be chalky when caught, but several were judged to be chalky

when landed 1–2 weeks later. Since it thus appeared that the condition probably developed post-mortem, a larger number of fish was examined in the summer of 1964 in order to obtain sufficient data to enable the preliminary finding to be confirmed or denied with certainty. Some 2000 halibut were taken during three separate fishing trips. None was found to be chalky at the time of catching, but from 35 to 70% of fish taken in different drags were judged to be chalky when landed 5–10 days later. A number of fish found to be not chalky after being held on deck (air temperature about 10°C) for 1 or 7 hr before being examined and iced, became chalky before being landed 6 days later.

Analyses of Halibut Caught by Dragging

Since, as mentioned above, preliminary observations in 1963 indicated the condition developed post-mortem, and since the change in appearance of the flesh seemed to be the result of some change in the state of the muscle proteins, the extractability of these proteins was investigated. Two samples from each fish were examined. One was taken and frozen when the fish was caught, the second when the fish was landed. In addition, since the pH of the muscle might be expected to exert an influence on the state of the protein post-mortem, the pH of the samples and their lactic acid content were determined. In view of Bailey's (1951) findings the dry weight and protein nitrogen contents of the samples were also measured.

Representative data obtained with 20 fish selected at random from a single drag are presented in Table I. On being landed, 12 of the fish were normal, eight chalky. It will be seen that there was a much more pronounced decrease in the extractability of muscle proteins in the chalky fish than in the others between the time of catching and landing. The flesh of the chalky fish had a pH below 6.0 when landed, whereas that of normal fish was 6.0 or higher. The lower pH of the chalky flesh at this time tended to be related to a higher concentration of lactic acid. There was also a tendency for the dry weight of chalky muscle to increase more than that of normal muscle during storage of the fish in ice. The increases in dry weight were roughly paralleled by an increase in the protein content of the muscle.

It is difficult to establish an exact muscle pH that would divide normal from chalky fish, for although the difference in appearance between normal and very chalky fish is readily apparent, there is a range in degrees of chalkiness that merges gradually into the normal appearance. However, on the basis of experienced grading, the dividing point appears to be very near pH 6.0. We have only found a few chalky fish with a muscle pH slightly above 6.0 (e.g. see section on Examination of Fish Caught by Longline). These latter fish were landed 2–3 weeks after being caught, and consequently it is possible their muscle pH may have reached a lower value than this at some time between catching and landing. Alternatively, it may also be that chalkiness develops at a pH a little above 6.0 if the fish is stored for a sufficiently long time in ice.

TABLE I. Relation between pH, lactic acid, protein solubility, and dry weight of halibut muscle and appearance of chalkiness.[a]

Condition when landed	Halibut muscle sampled										
	At catching						On landing 6 days later				
	Fish No.	pH	Lactic acid	Protein N		Dry weight	pH	Lactic acid	Protein N		Dry weight
				Total	Soluble[b]				Total	Soluble[b]	
				mg/g muscle		*% of wet wt*			*mg/g muscle*		*% of wet wt*
Normal	1	6.25	6.2	27.5	24.1	21.6	6.20	7.6	28.5	24.4	22.4
	2	6.16	6.2	27.5	23.9	22.6	6.02	6.6	27.2	24.0	23.5
	3	6.38	5.9	27.9	24.8	21.9	6.24	7.3	28.2	26.3	22.1
	4	6.21	5.4	27.4	25.4	22.0	6.00	7.6	28.4	23.2	22.8
	5	6.18	5.9	25.1	23.5	20.4	6.08	7.6	27.1	22.7	21.2
	6	6.29	5.3	25.0	23.3	19.8	6.30	6.3	26.4	22.3	19.6
	7	6.29	6.5	28.9	26.9	22.2	6.26	6.1	29.3	25.4	23.6
	8	6.33	6.2	30.1	28.0	23.4	6.16	7.5	30.4	26.1	22.8
	9	6.31	6.3	26.8	23.9	22.1	6.20	7.5	29.4	23.4	23.1
	10	6.34	5.1	29.0	26.7	22.9	6.28	7.1	28.9	24.6	23.1
	11	6.36	6.0	29.8	27.4	22.2	6.18	5.3	28.9	25.2	23.1
	12	6.23	6.5	28.6	23.7	22.1	6.10	5.8	28.2	25.4	22.4
Moderately chalky	13	6.15	6.5	25.6	23.3	21.3	5.93	7.7	28.4	19.1	22.4
	14	6.14	8.2	26.6	24.6	21.7	5.92	7.7	29.0	16.5	23.3
	15	6.19	6.6	28.8	27.0	22.3	5.85	8.1	32.1	17.9	25.0

Very chalky	16	6.20	6.0	29.1	25.2	23.3	5.71	9.3	29.7	14.5	23.6
	17	6.19	5.9	27.5	24.0	20.8	5.71	8.0	29.1	11.5	23.1
	18	6.25	6.1	27.9	24.8	22.3	5.70	8.2	30.4	11.5	25.1
	19	6.24	5.7	24.4	23.2	19.8	5.91	7.6	26.8	17.2	22.3
	20	6.20	5.3	27.4	22.8	22.1	5.78	8.3	30.1	11.7	24.4

Summary (average values)

	No. of fish										
Normal	12	6.28	6.0	27.8	25.1	21.9	6.17	6.9	28.4	24.4	22.5
Mod. chalky	3	6.16	7.1	27.0	24.9	21.8	5.90	7.8	29.8	17.8	23.6
Very chalky	5	6.22	5.8	27.3	24.0	21.7	5.76	8.3	29.2	13.3	23.7
Combined chalky	8	6.19	6.3	27.2	24.4	21.7	5.81	8.1	29.4	15.0	23.7

	Per cent protein extractable[b]	
	At catching	On landing
Normal	90.3	86.0
Chalky	90.8	51.0 (moderately 59.7) (very 45.6)

[a]Fish taken by dragging.
[b]In 5% NaCl, 0.02*M* $NaHCO_3$ solution.

Visual and Chemical Comparison of Changes in Samples in Following Thawing

As the flesh of normal and potentially chalky fish was indistinguishable at catching insofar as appearance was concerned and also insofar as pH and protein extractability of samples frozen at that time were concerned, but became distinguishable in these respects within a few days storage in ice (see previous two sections, and Table I), a comparison of thawed samples from normal and chalky fish was made with respect to appearance, pH, and protein solubility. Examinations of samples were made while they were still frozen, immediately after being thawed (8 min in air at 20°C), and after a further 2 hr storage at 20°C. Representative results are presented in Fig. 1 and 2 and Table II. It will be seen that while the samples frozen at catching were semi-translucent when thawed, that is, neither one appeared to be chalky, the sample from the potentially chalky fish became opaque within 2 hr, whereas that from the normal fish became only partly cloudy. The thawed sample from the chalky fish frozen at landing was opaque on thawing, while that from the normal fish frozen at the same time was partly cloudy. Neither of these samples changed appreciably in appearance during 2 hr following thawing. The marked

Table II. Changes in pH and in extractable protein in frozen normal and chalky halibut muscle on thawing

Condition at extraction	Frozen at catching			Frozen on landing		
	pH	Protein N soluble in NaCl solution[b]	Protein N soluble in KCl solution[c]	pH	Protein N soluble in NaCl solution[b]	Protein N soluble in KCl solution[c]
Normal fish[a]		*mg/g muscle*			*mg/g muscle*	
Frozen	6.31	26.8	5.2	6.25	25.3	4.4
Thawed (8 min, 20°C)	6.32	25.1	5.1	6.28	26.7	4.4
Thawed (2hr, 20°C)	6.33	23.0	4.8	6.24	24.3	4.1
Chalky fish[a]						
Frozen	6.18	25.2	6.0	5.74	13.4	2.6
Thawed (8 min, 20°C)	6.13	25.7	5.4	5.74	13.2	2.5
Thawed (2 hr, 20°C)	5.79	12.2	4.0	5.70	10.3	2.7

[a]Total protein N in the normal muscle was 28.9 and 29.3 mg/g when caught and when landed 6 days later, respectively; in the chalky muscle, 29.1 and 29.7 mg/g.
[b]5% NaCl, 0.02*M* $NaHCO_3$.
[c]0.025*M* KCl, 0.039*M* borate, pH 7.1.

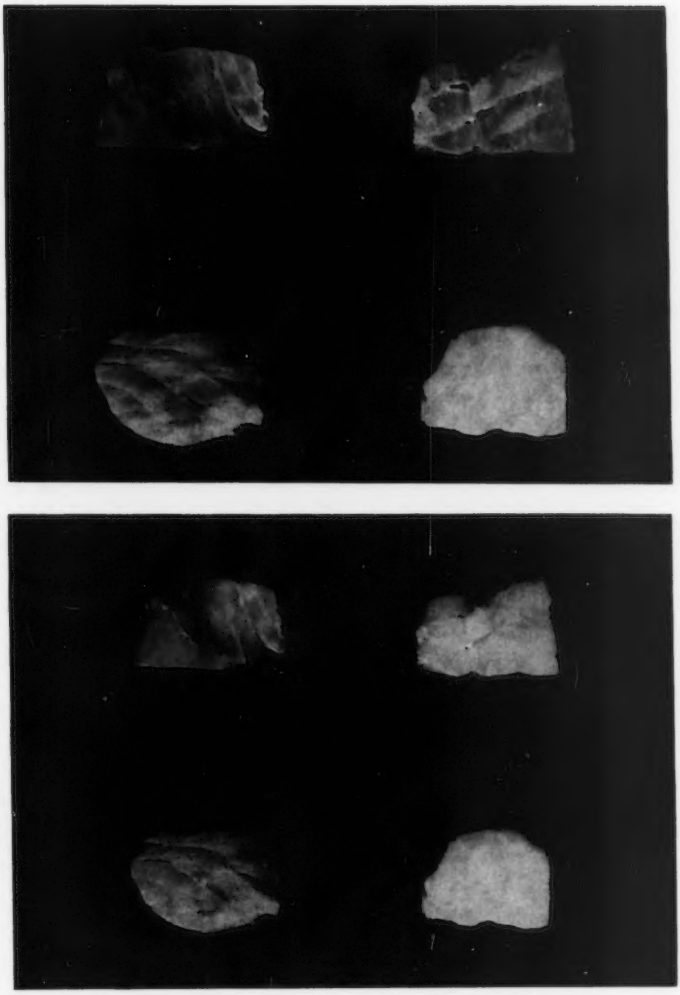

FIG. 1 (*top*). Samples photographed just after being thawed.

FIG. 2 (*bottom*). Same samples photographed 2 hr after being thawed. In each figure, the two samples at the left are from one normal fish, the two at the right from one chalky fish; the two at the top were frozen when the fish was caught, the two at the bottom when it was landed 6 days later.

change in appearance of the sample from the potentially chalky fish frozen at catching was accompanied by a marked decrease in pH and in extractability of both myofibrillar and sarcoplasmic proteins, the changes being similar in magnitude to the differences between the unthawed sample frozen on catching and that frozen ashore from the chalky fish (Table II). The changes in protein extractability that occurred in the other samples on thawing were very much smaller. It will be noted that there was a loss of extractability in both myofibrillar and sarcoplasmic proteins in the chalky fish between catching and landing.

Examination of Fish Caught by Longline

Samples of normal and of chalky fish caught by commercial longliners were obtained when the fish were landed in ice, 2–3 weeks after being caught. The chalky samples were analyzed for protein soluble in high-ionic-strength salt solution before being frozen and again afterward. The normal fish were analyzed for soluble protein without being frozen. Muscle protein and pH were also determined in all samples. Data for six normal and six chalky fish are presented in Table III. A very marked difference between normal and chalky fish in the extractability of their muscle proteins was obvious and of a similar magnitude to that found between normal and chalky trawled fish at landing. The difference was again found to be related to the pH of the muscle.

In the work described in the section on Analyses of Halibut Caught by Dragging, all samples had of necessity been frozen prior to analysis and the possibility existed that the decrease in muscle protein extractability observed in chalky fish might have occurred during freezing or subsequent frozen storage rather than during the preceding storage in ice. An examination of the data of Table III for the chalky fish will show that by far the greatest loss in protein solubility had taken place prior to freezing, although a further loss of about 7% took place when the muscle was frozen.

There was a tendency for the muscle pH of longlined fish to be higher than that of trawled fish at landing.

DISCUSSION

Recently it has been shown that the extractability and solubility of both sarcoplasmic and myofibrillar proteins of pig, ox, and rabbit muscle are markedly altered by post-mortem conditions of pH and temperature (e.g. Bendall and Wismer-Pedersen, 1962; Scopes, 1964; Scopes and Lawrie, 1963; Sayre and Briskey, 1963; Briskey and Sayre, 1964), and it seems clear from the present findings that alterations of a similar nature occur in halibut muscle, and that these changes are related to the post-mortem development of chalkiness. In addition, in the pig the changes in the proteins are accompanied by the development of a pale, soft, exudative condition of the muscle (Briskey, 1964), a condition resembling in some respects that observed in chalky halibut muscle. However, in the fish muscle it appears that a loss in solubility of myofibrillar

TABLE III. Soluble protein nitrogen and pH of normal and chalky muscle from halibut caught by longline.

	Fish No.	pH	Protein N		Per cent soluble		
			Total	Soluble[a]			
			mg/g muscle				
Normal fish	1	6.30	28.4	24.1	85.		
	2	6.70	26.5	26.0	98.		
	3	6.65	28.8	25.8	90.		
	4	6.34	28.3	24.4	86.		
	5	6.40	26.7	24.6	92.		
	6	6.40	28.8	25.4	88.		
Average values:		6.46	27.9	25.1	90.		
		Before freezing				*After freezing*	
						Protein N	
						Soluble[a]	Per cent soluble
						mg/g muscle	
Chalky fish	1	5.98	27.5	14.9	54.	12.0	44.
	2	5.90	30.6	13.9	45.	11.6	38.
	3	6.00	28.9	15.2	53.	13.0	45.
	4	5.80	28.8	13.2	46.	11.2	39.
	5	6.04	29.0	13.6	47.	12.1	42.
	6	5.74	30.6	11.0	36.	10.1	33.
Average values:		5.91	29.3	13.5	47.	10.8	40.

[a]In 5% NaCl, 0.02*M* $NaHCO_3$ solution.

protein may occur at a higher pH and lower temperature than has been observed in mammalian muscle, probably reflecting the greater lability of myosin in fish muscle (Connell, 1961).

Post-mortem losses in solubility of proteins soluble in low-ionic-strength salt solutions such as those observed in the present work, as far as we are aware, have not been recorded previously for fish muscle, and may be confined to certain species such as halibut in which the post mortem pH reaches relatively low values. However, although the usual observation has also been that there is no change in the solubility of these proteins in fish muscle during frozen storage (Dyer and Dingle, 1961), a decrease in their extractability has been observed in frozen lingcod (Tomlinson and Geiger, 1963), a species in which the lactic acid concentration does not apparently reach exceptionally high values (Tomlinson *et al.*, 1963).

While there is no direct proof that chalkiness is a result of a change in muscle proteins, it seems to be a reasonable explanation of the facts. The slightly "cooked" appearance of chalky muscle could be expected to be related to the loss in protein solubility which does occur, and the somewhat greater increase in dry weight (greater loss of water) by the chalky muscle during storage would be expected to be a result of the lower water-holding capacity of such muscle associated with its lower pH (Tarr, 1942) and decreased protein solubility (denaturation) (Connell, 1962). The softer condition, or reduced turgor, of the chalky muscle would also be a likely consequence of this. It is of interest that Tarr (1942) found the water-binding capacity of halibut muscle was at a minimum between about pH 4.5 and 6.0, the latter value being about the dividing point between normal and chalky fish observed in the present work. While the reduced turgor of the chalky muscle may contribute to the more ready separation of myotomes, it is perhaps more likely that physical disruption of connective tissue during rigor would be the cause (Reay, 1963), but it has also been suggested that a weakening of the bond between adjacent myotomes may be caused by a breakdown of connective tissue brought about by proteolytic enzyme action (Banks, 1962). This phenomenon in chalky halibut requires further investigation. A histological examination of various tissues and organs taken from normal and potentially chalky fish at the time of catching has not so far revealed any differences between them (J.R. McBride, personal communication).

The quantity of lactic acid found in fish muscle post mortem and consequently the pH, is related to the glycogen content of the muscle of the fish at hooking or netting, and this in turn is related to the state of nutrition of the fish (see review by Tomlinson and Geiger, 1962). Thus it would be expected that well-fed fish would be more likely to become chalky than would those less well fed. However, in the catching of halibut another possible influence may be of major importance. In the British Columbia fishery, dragging for halibut is illegal. The fish are caught commercially by longline in sets often of 24 hr duration and sometimes longer. Under such conditions, if the fish struggled until near exhaustion when hooked, they might have many hours in which to recover before being brought on board, for the majority of the fish are alive at this time. If this happened, it appears from the findings of Black *et al.* (1962) with rainbow trout that the lactic acid content of the muscle would be greatly reduced from its initial high value following struggling, while only a relatively small part, if any, of the muscle glycogen reserves would have been reformed. Such fish would then be less likely to become chalky than fish brought aboard soon after being hooked or netted, for their post-mortem muscle pH would usually be higher. In support of this view, there is some indication that in practice the incidence of chalkiness in longlined fish is lower than that found in the fish caught by dragging in the present work, in which drags were of 3 hr duration or less, and also that the muscle pH of longlined fish tends to be higher than that of netted fish — at least at the time of landing. This matter requires clarification, for it may be that dragging will take heavily fed

fish that could not be caught by longline. It should be pointed out that while no fish has been found to be chalky when brought on board by dragging, it appears possible that if a fish died soon after being hooked by longline, chalkiness might develop by the time the fish was brought on board if the time it was left in the water was unduly prolonged.

The present findings with regard to protein and water content of halibut muscle show that there is not necessarily any difference in this respect between normal and potentially chalky fish at the time of catching. Also, although there is a distinct tendency for the water content to decrease and for protein content to increase more in chalky than in normal fish during storage in ice, the ranges of their protein and water contents overlap following storage. The present findings are thus in accord with those of Patashnik and Groninger (1964) who found the protein and water content of chalky fillets to fall within the normal range for halibut. On the other hand, Bailey (1950, 1951) found that chalky flesh can be unusually low in water and high in protein and oil. While a greater water loss by chalky fish during storage in ice, such as that found in the present work, may have influenced Bailey's findings, in some circumstances, such as those of relatively prolonged differences in feeding, differences such as those observed by Bailey could occur (e.g. Ironside and Love, 1958). These would be expected to be accompanied by a higher muscle glycogen content in the better fed fish (which would also have the higher protein and oil content) and hence greater susceptibility to chalkiness.

Further investigations, particularly of the matters indicated above to be in need of clarification, and of the possible usefulness of freezing at sea as a means of reducing losses through chalkiness, are being undertaken.

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Factors Affecting Stream Location, and Timing and Intensity of Entry by Spawning Kokanee (*Oncorhynchus nerka*) into an Inlet of Nicola Lake, British Columbia[1]

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ABSTRACT

Diel fluctuations in onshore–offshore movement of mature kokanee occurring at the northeast end of Nicola Lake adjacent to a spawning stream were related to differences in wind action, which thereby affected daily numbers of kokanee entering this stream. Nasal occlusion experiments indicated that olfaction was used in location of the stream by kokanee. Entry into the stream occurred largely from dusk to midnight and appeared to be related to changes in light intensity rather than temperature. Average length of spawning kokanee (sexes combined) ranged between 225.9 and 289.9 mm from 1958 to 1963 with marked annual differences. Most kokanee spawned in their third year (2 +) although some were in their second and fourth years (1 + and 3 +). About equal numbers of both sexes occurred in the spawning population; males were more abundant at the onset of the migration while females were predominant towards the end.

INTRODUCTION

Although there has been a prodigious amount of work done on many facets of the spawning migration of anadromous sockeye salmon (Wilimovsky and Freihofer, 1957), surprisingly little effort has been directed towards such migration in the strictly freshwater form — kokanee. Most studies on spawning kokanee have described the morphology, meristics, abundance, age, growth, sex ratio, and other characteristics of the migrating population (Vernon, 1957; Tokui, 1960, 1961; Kurohagi and Sasaki, 1961; Brunson *et al.*, 1952; Allison, 1958). The elucidation of factors which may control stream location, timing and intensity of stream entry, and related features of the spawning migration itself has rarely been attempted despite the fact that these aspects of migration should be more amenable to investigation in kokanee than in their anadromous counterparts.

The present study is primarily concerned with the spawning *migration* of kokanee from a lake into and within an inlet stream. Relevant characteristics of the spawning population are presented only secondarily.

DESCRIPTION OF THE STUDY AREA

The spawning migration of kokanee was studied at Nicola Lake in south-central British Columbia (50°10′N, 120°30′W). Some features of the limnology of Nicola Lake have been described by Rawson (1934) and Northcote

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et al. (1964). The lake (Fig. 1), 21 km in length, has a surface area of 2516 ha, a maximum depth of 55 m and a mean depth of 23.5 m. Additional limnological data are given in Table I.

Of the several streams entering Nicola Lake only two, the Nicola River and Moore Creek, are known to serve as significant spawning areas for kokanee. No kokanee were seen in Quilchena Creek in 1959. The Nicola River, draining several lakes to the east, is by far the largest tributary, with a maximum discharge in the spring of over 11 m^3/sec but dropping to about 1 m^3/sec by late August. Nicola River probably supports a larger spawning population of kokanee than does Moore Creek where this study was conducted. Moore Creek, entering the northeastern end of the lake, drains a series of small lakes most of which have been dammed to provide water for agricultural purposes. After maximum discharge in May (over 4 m^3/sec in 1959), the creek gradually drops throughout summer to slightly less than 0.1 m^3/sec by late August on dry years. The majority of kokanee spawn in the lower 2 km of Moore Creek where it ranges between 5 and 8 m in width. A small sand delta has formed at the mouth of Moore Creek which opens near the center of a long sand and gravel beach extending across the entire head of Nicola Lake (Fig. 1).

METHODS

Shoreward movement of kokanee onto the beach at the head of Nicola Lake was recorded by series of gill nets set at approximately 90° to the shore. For most sets, 4 monofilament (0.20-mm) nylon nets of 51-mm stretched mesh, each about 15 m long and 2.5 m deep were joined end to end onto two similar nets 7.5 m deep. Such a gang set with the 2.5-m net onshore sampled fish at all depths in the first 90 m off the shore. The gang of nets was fished for 2- or 4-hr periods and then was replaced with a duplicate set. The netting series was continued for 24 hr or longer to provide information on diel distribution and movement of kokanee in near-shore waters. Additional records of fish distribution in this region were obtained with a 50 Kc/s Furuno F-701 recording echo sounder as described by Northcote *et al.* (1964).

Standard limnological determinations were made at Station 1 on the lake (Northcote *et al.*, 1964). Stream temperature records were obtained with a continuous temperature recorder. Stream water level was measured with a staff gauge located about 300 m upstream from the mouth of Moore Creek. Surface illumination at the creek mouth between evening and morning was measured with a Photovolt Model 514 M photometer fitted with phototube C and a scotopic (human eye) correction filter as described by Northcote (1962).

Entrance of kokanee into Moore Creek and movements within the creek were recorded in diagonal fence traps similar to the design of Shetter (1938). Trap 1 was located 15 m upstream from the creek mouth while Trap 2 was installed about 300 m farther upstream. Both traps and fences were fitted with 19-mm square mesh regalvanized wire screening.

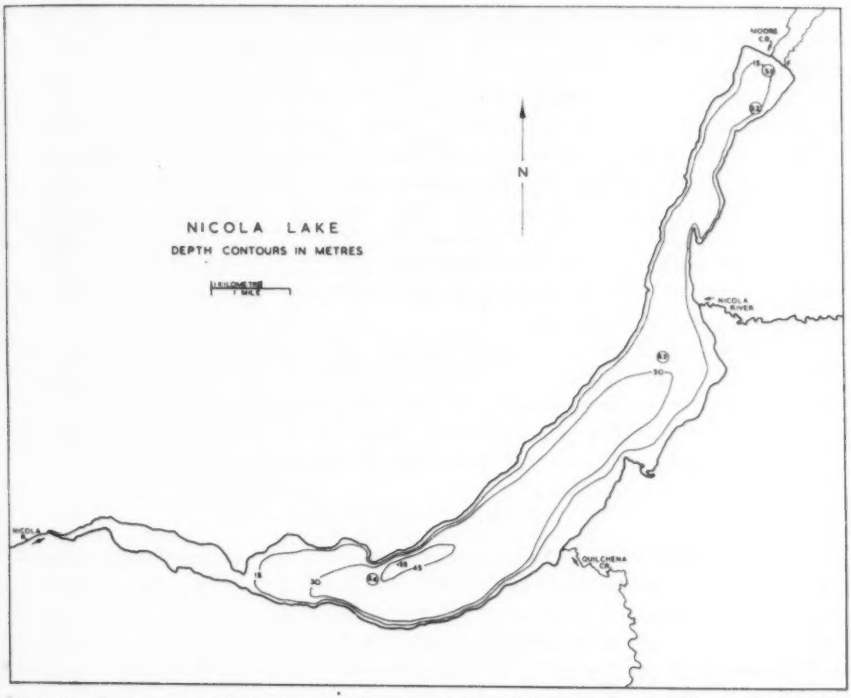

FIG. 1. Contour map of Nicola Lake showing location of limnological and gill netting stations (S1–4).

TABLE I. Some limnological characteristics at Station 1 of Nicola Lake, British Columbia.

Year	Mean temperature 0–15 m	Mean oxygen 0–15 m	Mean secchi disc reading	Mean zooplankton volume[a]
	°C	*mg/litre*	*m*	*cc*
1958	19.1 (Aug. 25)	6.0 (Aug. 25)	1.7 (Aug. 8–Sept. 7)	1.5 (0–18 m, Aug. 6) 1.2 (0–18 m, Aug. 20) 1.1 (0–17 m, Sept. 15)
1959	15.8 (Aug. 24)	8.9 (Aug. 31)	1.7 (Aug. 10–Sept. 20)	2.6 (0–15 m, July 31) 3.3 (0–15 m, Aug. 14) 3.2 (0–15 m, Aug. 17)
1961	19.1 (Aug. 27)	8.5 (Aug. 19)	3.5 (Aug. 18–30)	2.7 (0–14 m, Aug. 18) 2.3 (0–14 m, Aug. 19)
1962	16.9 (Aug. 25)	10.5 (Aug. 26)	2.2 (Aug. 25–27)	No data
1963	17.9 (Aug. 25)	10.0 (Aug. 25)	2.4 (Aug. 25)	No data

[a]Settled volume of plankton (largely zooplankton) taken in vertical hauls with a number 10 mesh Wisconsin net with 25-cm mouth opening; mean based on triplicate hauls, usually; depths sampled: 0–18 m in 1958, 0–15 m in 1959, 0–13.5 m in 1961.

Kokanee that moved from Nicola Lake into Moore Creek on any particular evening either entered Trap 1 or remained in a pool immediately below its fence until dawn when they returned to the lake. During the 1959 spawning run, kokanee not entering the trap were seined from this pool when the trap was emptied and were passed upstream after being recorded. However, in 1958, kokanee were not seined from the pool until late in the spawning run.

Traps were checked and emptied of fish several times during a 24-hr period, usually at 1600, 2000, 2200, 2400 and 0600 hr. All fish contained in the trap or seined from the pool immediately below Trap 1, between the hours of 1600 and 0600 (the following morning), were considered as 1 day's catch. Hourly counts of kokanee entering Traps 1 and 2 were also made several times during the spawning migration.

In 1958, length, sex, and scale samples were taken from all fish entering Moore Creek (Trap 1) while in 1959 only periodic samples were taken although the majority of fish in the spawning migration were counted. On most subsequent years, samples were obtained from the spawners entering Moore Creek but no attempt was made to enumerate the migrations.

ONSHORE MOVEMENT

In late August 1959, gill net catches at several stations indicated a gradual concentration of maturing kokanee near the head of Nicola Lake as well as marked diel onshore–offshore movement of these fish at the northeast end of the lake. Similar patterns of movement were noted at about the same time in 1962 and again in 1963 when the most complete series of data was obtained (Fig. 2).

In 1963, the netting series was conducted about 75 m northwest of Moore Creek mouth while on previous years the nets were set about 200 m away. These and other netting data indicated that onshore movement of kokanee was not restricted to the immediate vicinity of the creek mouth but occurred along the whole northeast margin of the lake.

Because no attempt was made to record location of kokanee along the length of each net, their horizontal distribution shown in Fig. 2 within each 15-m interval has been evenly spaced. However, the 7.5 m deep offshore nets were marked into five equal divisions vertically and the four nets fished between shore and 60 m were marked into upper and lower halves. It was possible, therefore, to record the vertical distribution of the kokanee caught in the nets and this has been indicated in Fig. 2.

Relatively few kokanee were captured in the shallow shore zone (0–60 m), during the mornings and afternoons, but considerable numbers were taken there during the evenings (1800–2000 hr) and throughout the remaining periods of darkness. By early morning no kokanee were captured in the net closest to shore and very few were evident in any of the shallow zones.

In 1962 and 1963, echograms were made between Station 1 and the onshore end of the gill net sets to obtain more information on movement of fish onshore. Ability of the sounder to record individual mature kokanee and pea-

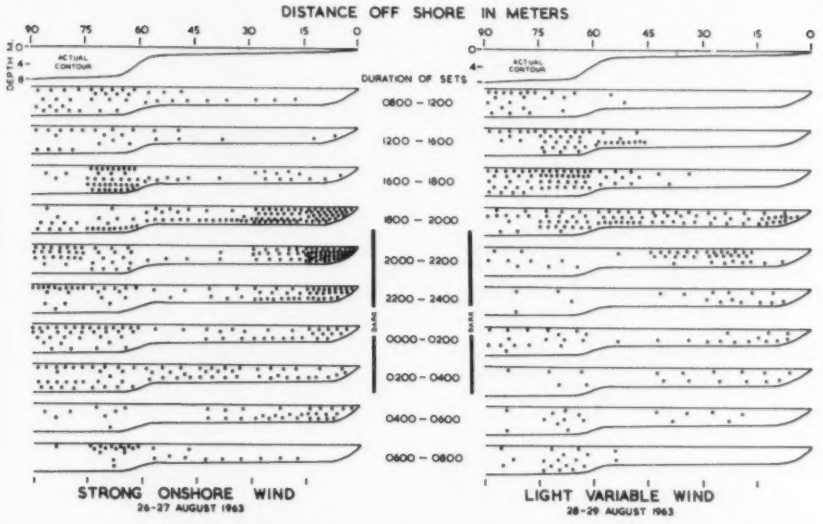

FIG. 2. Distribution of mature kokanee (black dots) in gill nets set off beach at northeast end of Nicola Lake (shape of contours showing kokanee modified slightly to facilitate plotting; see text for further details).

mouth chub was demonstrated previously (Northcote *et al.*, 1964). Between 50 and 65% of the several hundred fish taken in each 24-hr netting series were kokanee so they were probably well represented in the flecks indicating fish (Fig. 3 and 4).

The most frequent series of echograms was made on August 28–29, 1962 (Fig. 3). This series shows the rise and shoreward spread of fish with the approach of dusk and ascent of 1 lux isopleth to the lake surface between 1820 and 1935 hr. However at dawn the concentration of marks (indicating fish) moved away from the shore and surface layers and descended to levels where illumination was in the order of 1 lux or less. A similar sequence of movement was indicated on echograms (Fig. 4) made along the net sets depicted in Fig. 2.

Evidence from netting data in 1959 suggested that mature kokanee moved into shallow regions along the whole beach at the northeast end of the lake much earlier in the evening and in greater numbers on days when there were strong onshore winds than on calm days or those with light variable winds. Because of netting difficulties in rough weather it was not possible to complete a 24-hr series in 1959 but this was accomplished in 1963 (Fig. 2).

During the 24-hr netting period with strong onshore winds a totalizing anemometer mounted about 2 m above the ground a few meters from the shore end of the net recorded an average velocity of 60.5 km/hr. Over the same period a day later the average velocity was only 3.7 km/hr and frequently the lake was either calm or subjected to light offshore breezes. Throughout daylight hours kokanee were always present in shallow water within 30 m of shore

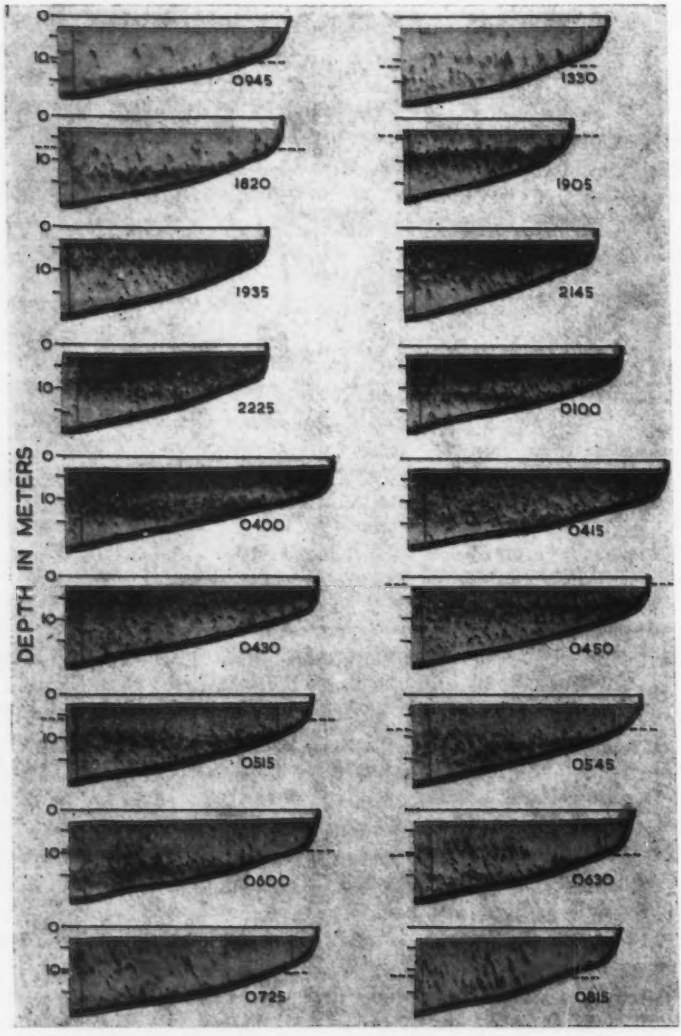

FIG. 3. Echo sounder traces between Station 1, Nicola Lake, and the shore adjacent to the mouth of Moore Creek on August 28–29, 1962. Uppermost 2.5–3 m not recordable on sounder; broken line shows level of 1 lux illumination.

during the windy period but were absent from this area during the calm period (Fig. 2). Furthermore the numbers of mature kokanee taken onshore were much greater on the evening with strong onshore winds than on the calm evening. Offshore movement also appeared to begin later on the windy morning. Echograms made at the same time as the netting series also suggested that fish, many of them undoubtedly kokanee, were distributed closer to shore

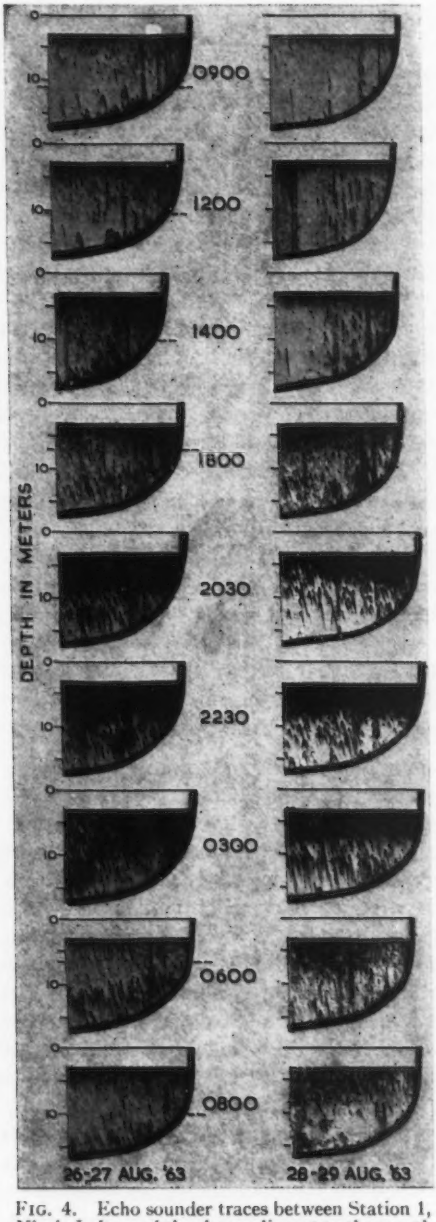

FIG. 4. Echo sounder traces between Station 1, Nicola Lake, and the shore adjacent to the mouth of Moore Creek in August 1963. Uppermost 2.5–3 m not recordable on sounder; broken line shows level of 1 lux illumination.

on the windy day, that they attained greater concentration in onshore waters at night, and that they moved offshore later the following morning (Fig. 4). Unfortunately failure of the underwater photometer prevented a comparison of subsurface light intensities between the 2 days. Both days had broken cumulus cloud cover (average 39% on August 26–27 and 28% on August 28–29) so any differences in subsurface light intensity would have been largely the result of wave action.

In mid September 1958, kokanee observations were made during several 24-hr periods at several stations along the beach within a 300-m radius of Moore Creek mouth. During daylight hours sections of shallow water were observed from vantage points with binoculars while at night observations were made using short bursts of light from a spotlamp, or with infrared viewing apparatus as described by Northcote (1962). No kokanee were seen onshore until dusk (1800–1830 hr). Thereafter groups containing up to 10 fish were noted at all stations, often less than 1 m away from the lake edge. These groups of kokanee were usually swimming parallel to the shore both towards and away from the creek mouth. Although there were greater concentrations in the immediate vicinity of Moore Creek mouth, groups were frequently seen as far as 300 m away. On windy evenings, kokanee were so close to shore that occasionally they were tossed up onto the beach with incoming waves and had to skitter over a short distance of damp sand and gravel to return to the water. Kokanee were not observed in the shallow water along the lake edges after dawn.

ENTRANCE INTO SPAWNING STREAM

Location of Stream Mouth

Additional observations as described above were made on the movement of kokanee at the mouth of Moore Creek. On calm evenings Moore Creek water could be traced thermally as a fairly discrete entity for several meters into the lake. At such times small groups of kokanee, swimming more or less parallel to the shoreline, were repeatedly observed to turn abruptly upon reaching the influence of the creek and move up it to the shallow delta at its mouth where they usually stopped briefly before entering the creek itself. Occasionally individuals which veered out of the path of creek water within the lake appeared to circle about until they had relocated its influence.

In an attempt to determine the importance of olfactory and visual stimuli in aiding kokanee to locate the spawning stream, a number of short-term experiments was conducted on fish that had entered the stream and moved into Trap 1.

On September 12, 1958, near the middle of the spawning migration, 40 kokanee (20 males, 20 females) were taken from Trap 1 in the morning and marked by clipping off the adipose fin. Both nasal rosettes were heat cauterized in half of these fish (10 males, 10 females) while the others were not treated except for dipnet handling. All fish were released at midday at the surface of Nicola Lake about 150 m off the mouth of Moore Creek. A total of nine

fish (two males, seven females), all noncauterized, were recovered in Trap 1 by September 22 when the migration was nearing completion. A similar experiment was started on September 17, 1958, using 100 kokanee (50 males, 50 females) marked by removing the posterior portion off the dorsal fin. Both nasal rosettes were cauterized on half of the fish before their release. By September 22 when Trap 1 was removed, 13 fish, all noncauterized (seven males, six females) had been recovered there from this group. Several cauterized and noncauterized kokanee were held without mortality for several days in a holding box in Moore Creek during the period when experimental fish were released.

Another experiment was carried out early in the 1959 spawning migration. On August 22, kokanee entering Moore Creek were marked by removing the distal half of the adipose fin and treated as follows: 50 had both nostrils occluded with latex (technique modified from Gunning, 1959), 50 had the left nostril occluded with latex, 25 had both nasal rosettes heat cauterized, 25 had the left nasal rosette cauterized, 50 had vision impaired or destroyed by formalin injection into the pupil, and 50 were untreated. All fish were released at midday about 50 m off the mouth of Moore Creek. Of the 250 fish released, 30 were recovered in Trap 1 until its operation was discontinued on September 22. These consisted of 26 untreated fish, two with one nasal rosette cauterized, and two with both nasal rosettes cauterized (cauterization had not completely destroyed the rosettes of these latter two fish). Twenty of the untreated fish were recovered within 8 days after release. Groups of kokanee with one or both nostrils occluded with latex showed no mortality or apparent ill effects when held for several days in cages both in the lake and in Moore Creek. However, nostril occlusion and particularly cauterization may well have a traumatic effect on the experimental fish, thereby inhibiting or delaying their migratory response.

Diel Periodicity

Throughout the spawning migration in 1958 and 1959, the temporal pattern of catches in Trap 1 showed that most kokanee entered Moore Creek between dusk and dawn. On several occasions in each year the trap was checked at hourly intervals to study diel periodicity of kokanee entrance in more detail (Fig. 5). The majority of fish entered Moore Creek early in the evening so that the migration was virtually ended by midnight. The sudden onset of migration was coincident with rapid decline in light intensity at dusk. There were no marked changes in stream temperature during this period.

More precise information on commencement of migration in the evening was recorded by observing passage of fish over a counting strip placed on the bottom of the stream mouth. Counts were made from a vantage point away from the edge of the stream until late evening when infrared viewing apparatus was used. By starting observations well before the onset of migration, entrance of the first kokanee into the stream could be recorded.

Representative data for two evenings late in the 1958 spawning migration and another two early in the 1959 run are given in Fig. 6. Migration commenced

each evening about the time when light intensity fell to 100 luxes. Both the onset of migration and decline of light intensity below 100 luxes occurred nearly an hour earlier by mid September.

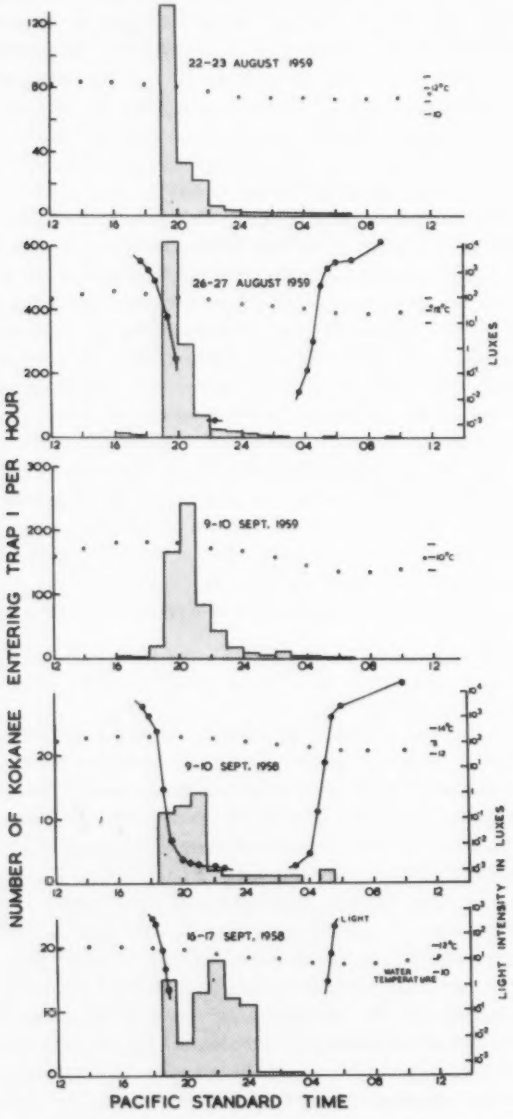

FIG. 5. Diel periodicity in spawning migration of kokanee into Moore Creek, Nicola Lake, British Columbia.

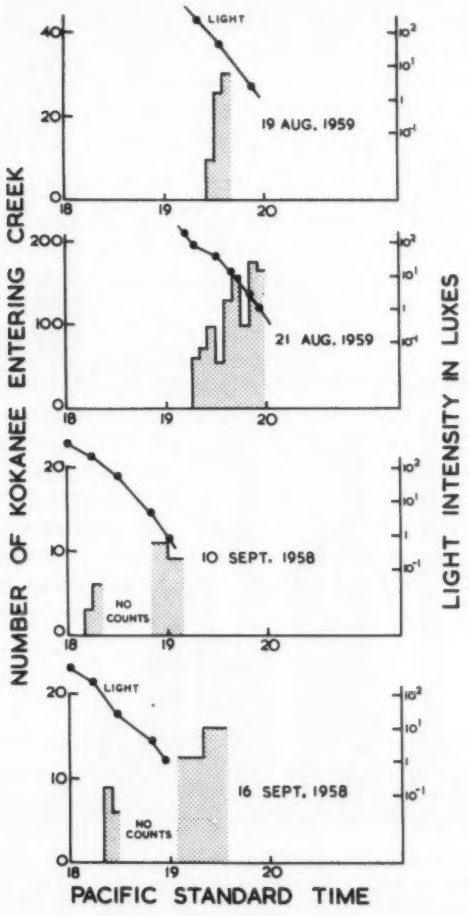

FIG. 6. Commencement of kokanee spawning migration at Moore Creek, Nicola Lake, in relation to light intensity.

DAILY FLUCTUATIONS

Large fluctuations occurred from day to day in the numbers of mature kokanee entering Moore Creek (Fig. 7). These changes did not appear to be related to the rather small variations in stream discharge either in 1958 when stream level was recorded daily or in 1959 when level was recorded occasionally. Among the environmental factors considered which conceivably might affect daily intensity of the migration, creek temperature, and especially wind-induced circulation at the creek mouth appeared worthy of more detailed analysis. Unfortunately in 1958 some of the kokanee entering the creek each evening were not counted until late in the migration (see Methods) and records

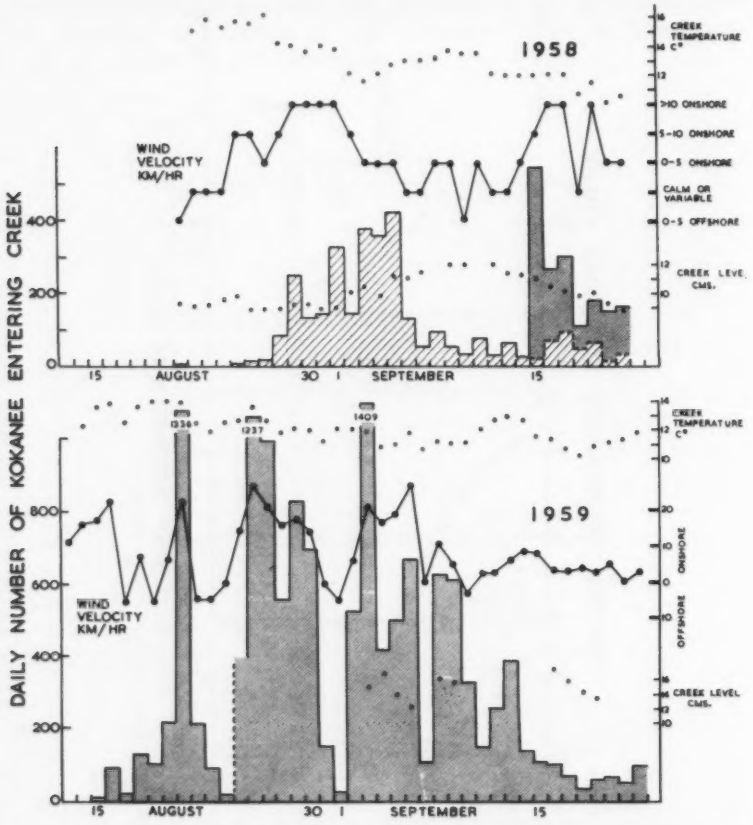

FIG. 7. Daily fluctuations in mature kokanee entering Moore Creek. Light hatching represents fish taken in Trap 1 only; heavy hatching gives total for Trap 1 plus those seined from pool immediately below; wind velocity averaged for 1200–2400-hr period.

on wind velocity were not as complete as desired. Nevertheless a tendency for kokanee to enter the creek in greater numbers on days when there were moderate to strong onshore winds is suggested (Fig. 7). This association is more obvious in 1959 when accurate wind velocities were measured between midday and midnight with a totalizing anemometer and when the majority if not all kokanee entering the creek each evening were recorded. The 1200–2400-hr period was chosen for averaging wind velocity because it was during this time interval that kokanee were located immediately offshore and began movement towards shore at dusk. However, the summer and autumn wind pattern on Nicola Lake was such that strong winds usually continued for 24 hr or longer. Rarely were periods of strong wind restricted to the early morning.

To examine the relationship between daily fluctuations in number of kokanee entering the spawning stream and changes in both stream temperature and wind action, data for 36 days (August 15–September 20, 1959) were treated in a multiple regression analysis. Observations on August 25 were excluded since many unrecorded kokanee passed Trap 1 through a large temporary hole in the fence.

Onshore and offshore wind velocities were given positive and negative values, respectively, and 10 units were then added to make all variates positive. Stream temperature was expressed in C° change in the daily maximum, 3 units being added to all variates to make them positive. The daily number of kokanee entering Moore Creek was coded so that 0–50 fish were given a value of 1, 50–100 a value of 2, and so on. The R value for the whole regression, 0.712, was significant ($p < 0.01$) as was the partial regression coefficient for wind (0.725). That for temperature (–0.109) was not significant ($p > 0.3$). If the regression excluded the beginning and concluding days of the migration observed (August 15–16 and September 18–20) when few mature kokanee might have been available to enter Moore Creek regardless of stimulation by onshore winds or other factors, the R value for the whole regression was increased (0.809). Apparently then, during the active period of the spawning migration, the number of kokanee entering this stream daily may be strongly influenced by the intensity of onshore wind at the stream mouth but not by changes in stream temperature.

MOVEMENT WITHIN STREAM

Diel Periodicity

Upstream movement of mature kokanee within Moore Creek was recorded in detail over several 24-hr periods. Representative data are shown in Fig. 8. In contrast to their entry into the creek (Fig. 5), kokanee within the creek began to move upstream early in the evening and continued to do so well into morning. Upstream movement of kokanee within the creek was not restricted to a short, intensive period prior to midnight as was characteristic of their entry. Early in the season the period of upstream movement was short but later on it extended to well over 12 hr (Fig. 8).

Extent and Rate of Movement

Periodically throughout the spawning migration in 1959, groups of kokanee were marked distinctively by fin-clipping at Trap 1. Recovery of these at Trap 2, 300 m further upstream, gave information on extent and rate of upstream movement. Although more fish apparently remained below Trap 2 than moved above it, even early in the migration, there was a gradual decrease in the per cent recaptured at this trap as the migration advanced (Table II). The actual sequence of recoveries at Trap 2 did not suggest that there were large numbers of marked fish from the later releases which still would have been recovered had the traps been operated beyond September 23. Indeed

during the early part of the migration, kokanee marked at Trap 1 were not captured at Trap 2 until 4–5 days afterwards whereas later in the migration many individuals were taken at Trap 2 the day after being marked.

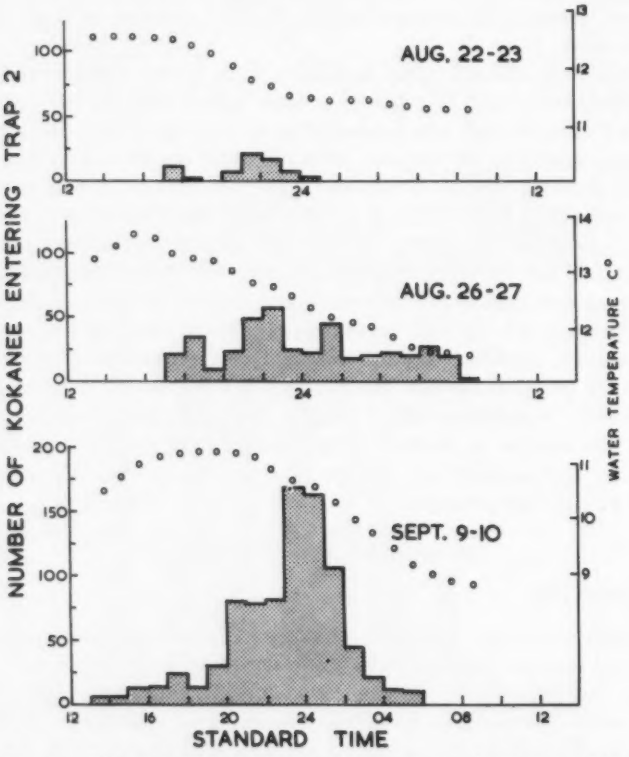

FIG. 8. Diel periodicity of upstream movement of kokanee within Moore Creek for three periods of the 1959 spawning migration (see Fig. 5 for light intensities).

TABLE II. Number of mature kokanee marked at Trap 1 on Moore Creek in 1959 and subsequently recaptured at Trap 2.

Date marked	Number marked	Number recaptured	Per cent recaptured
Aug. 15–18	338	153	45.3
Aug. 19–21	670	251	37.5
Aug. 22–27	392	137	34.9
Aug. 28–30	409	131	32.0
Sept. 5	100	10	10.0

CHARACTERISTICS OF THE SPAWNING POPULATION

LENGTH

In 1958 and 1959, measurements of mature kokanee were made throughout the run from representative samples in Trap 1 (Fig. 9). In other years samples of mature kokanee were taken in August by seining at the Trap 1 site or by gill nets set near the creek mouth. From 1957 to 1959 all spawning kokanee were less than 275 mm in length with averages for the samples well below 240 mm (Table III). In 1960 there was a marked increase in the length of kokanee sampled from the spawning migration; indeed most were larger than 275 mm. Since 1961 there has been a slight decrease in length of the kokanee sampled with some comparable in size to those in 1958 or 1959. Most however were still larger than any recorded between 1957 and 1959. As will be noted later, no marked change has apparently occurred in sex or age composition of the spawning population.

AGE

Scales from kokanee in the 1958 spawning migration proved difficult to age because of partial "checks" which could be interpreted either as annuli or as summer checks. Consequently in August 1959, samples from all size groups of kokanee in Nicola Lake were collected by dynamite explosions at several depths near Stations 1 and 3. All scales from the widely ranging length groups (Fig. 10) were closely examined. The smallest size group, ranging between 38 and 55 mm fork length, had scales with only 3–7 circuli and no annulus indicating they were probably progeny of the 1958 spawners. These were 0 + fish about 10 months in age from time of fertilization. The next size group (76–163 mm) all had 11 or more circuli with a single annulus somewhere between 7–13 circuli; these were completing their second year or 1 + fish. All kokanee over 180 mm had scales showing at least 2 annuli. Many of the scales, especially from the second and occasionally from the third size group had 2 or 3 closely spaced circuli at the scale margin suggesting a summer check.

On the basis of the material summarized above, ages of the various size groups in the August 1959 collection were designated as shown in Fig. 10 and used to interpret ages from kokanee scales in both the 1958 and 1959 spawning migration. Most spawners in these years apparently were completing their third year (2 +) although there were somewhat more fish in the 3 + age category in 1958 and in 1959. Immature 1 + and 2 + age group kokanee were taken in gill nets set at Station 1 well after the spawning migration in 1959 (Fig. 10). Although no scales were taken from kokanee netted at Station 1 in April 1960, their length distribution (Fig. 10) indicates that they would have been comprised largely of fish early in their third and fourth year (2 + and 3 +). Furthermore their size distribution was already comparable to that attained by fish netted throughout June at Station 1 in 1959, indicating that spawners in the 2 + age group for 1960 would be larger than those of the

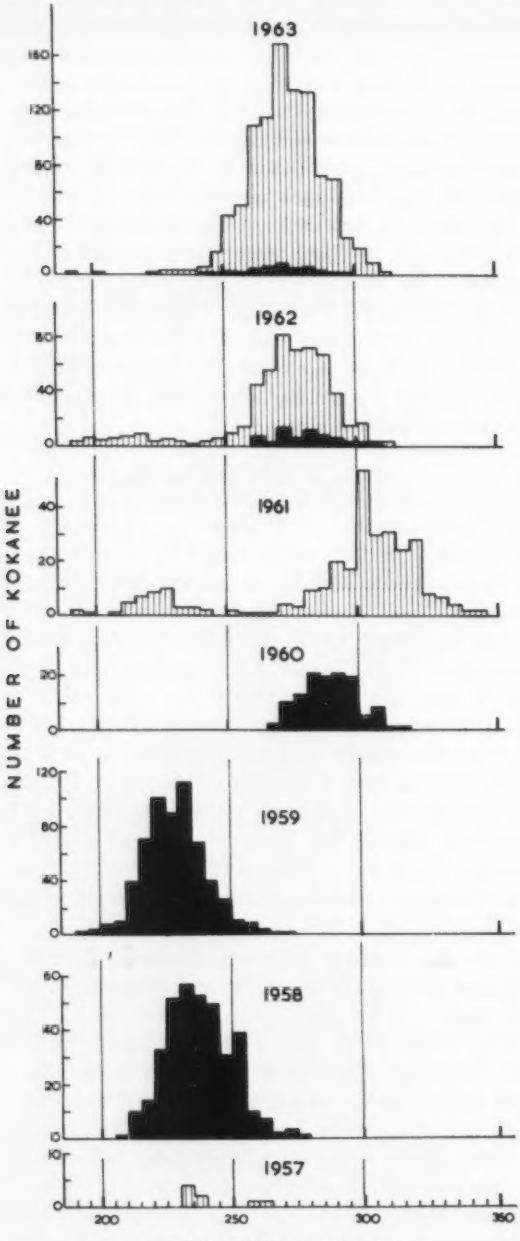

FIG. 9. Length–frequency distributions for mature Nicola Lake kokanee. Dark histograms represent fish captured in Trap 1, Moore Creek; light histograms show fish captured in standard gill net sets near the creek mouth.

TABLE III. Length distribution of mature kokanee sampled from Moore Creek or near mouth of Moore Creek, Nicola Lake.

Year	Method of capture	Sex	Number sampled	Average fork length
				mm
1957	Gill nets, Moore Creek mouth	Combined	8	234.4
1958	Trap 1, Moore Creek	Male	166	236.0
		Female	194	229.6
		Combined	360	232.5
1959	Trap 1, Moore Creek	Male	180	227.1
		Female	216	225.0
		Combined	396	225.9
1960	Seined, Trap 1 site, Moore Creek	Male	41	288.4
		Female	78	281.5
		Combined	119	283.9
1961	Gill nets, Moore Creek mouth	Male	309	283.3
		Female	259	297.9
		Combined	568	289.9
1962	Seined, Trap 1 site, Moore Creek	Male	42	278.1
		Female	19	272.9
		Combined	61	276.5
	Gill nets, Moore Creek mouth	Male	158	261.1
		Female	104	273.0
		Combined	262	265.8
1963	Seined, Trap 1 site, Moore Creek	Male	21	269.1
		Female	19	269.8
		Combined	40	269.4
	Gill nets, Moore Creek mouth	Male	274	275.4
		Female	175	272.1
		Combined	449	274.1

previous year, as indeed was the case (Fig. 9 and 10). Although spawning kokanee were much larger from 1960 to 1963 than those in 1958 or 1959 there was no indication of a change in age class structure; the majority of spawners were nearing the end of their third year. All three age groups in the spawning population, 1 +, 2 +, and 3 +, were comprised of considerably larger fish in 1961 and 1962 than in 1958 or 1959.

SEX RATIO

In 1958 when all kokanee entering the mouth of Moore Creek were examined there was no significant departure ($p > 0.5$) from a 1:1 ratio in the total number of males and females taken at Trap 1 (Table IV). However, males were more abundant at the start of the migration while females were predominant towards the end. A similar trend was evident at Trap 1 in 1959 although few kokanee were sampled and the sex could not be determined

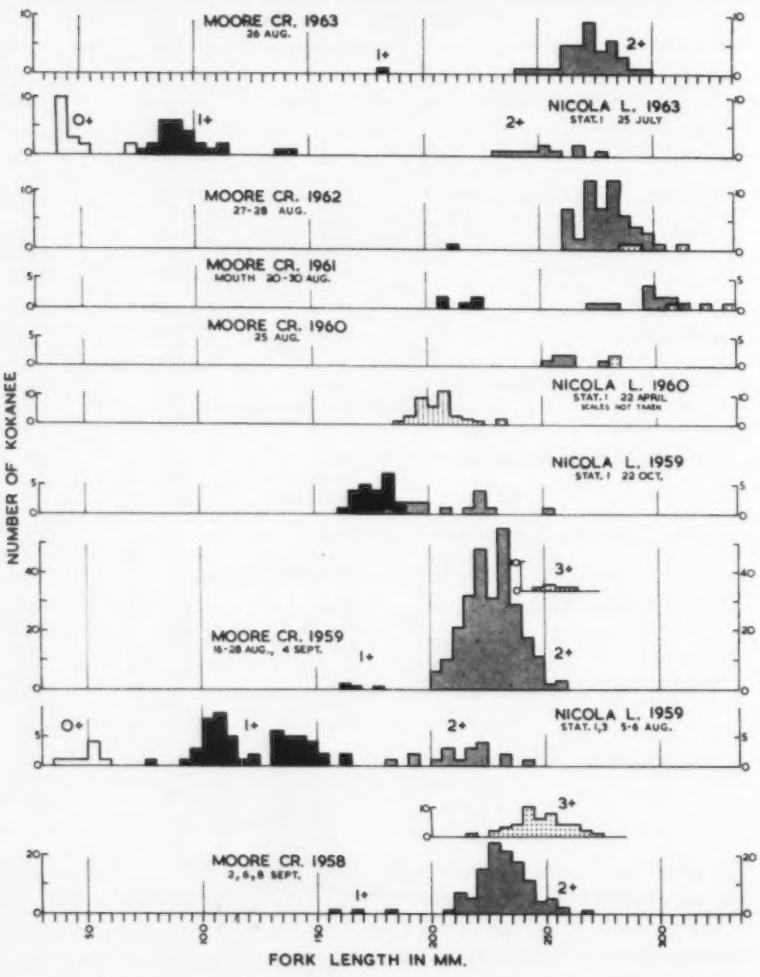

FIG. 10. Age and length-frequency for Nicola Lake kokanee (see text for details).

externally on many of the early migrants. On the other hand at Trap 2 in 1959 there was no suggestion of a decrease in the proportion of male kokanee as the migration advanced. There was a higher ratio of males to females during each of the periods and the total ratio showed a significant departure ($p < 0.001$) from 1:1. Undoubtedly many of the fish on which sex could not be determined were females, especially in the early period, but even if all these were assumed to be females there would still have been a preponderance of males. Females may show a greater tendency to spawn in the lower sections

TABLE IV. Percentage of male and female mature kokanee entering Moore Creek during several periods of their spawning migration in 1958 and 1959.

Year	Capture site	Time period	Number examined	Males	Females	Sex unknown
1958	Trap 1	Aug. 20–30	511	53.2	46.0	0.8
		Aug. 31–Sept. 9	2104	52.2	46.7	1.1
		Sept. 10–21	1947	46.6	52.1	1.3
		Total:	4562	49.9	48.9	1.2
1959	Trap 1	Aug. 17–28	360	43.1	38.9	18.0
		Sept. 4–17	80	33.7	65.0	1.3
		Total:	440	41.4	43.6	15.0
1959	Trap 2	Aug. 20–30	1346	56.1	27.0	16.9
		Aug. 31–Sept. 9	2397	52.9	43.6	3.5
		Sept. 10–20	1538	55.5	44.1	0.4
		Total:	5281	54.5	39.5	6.0

of the stream than do males, especially towards the latter portion of the spawning migration, while the males may move farther upstream.

DISCUSSION

At the north end of Nicola Lake mature kokanee moved onshore late each afternoon during the spawning season and in the evening many entered Moore Creek where they subsequently spawned. A similar pattern of movement also occurred near the mouth of Nicola River, the other spawning site for kokanee, but it was not studied there in detail.

The onset of stream entry by mature kokanee appeared to be timed by light intensity and to be unrelated to temperature changes. Kokanee began entering the stream each evening when surface light intensity fell below about 100 luxes and at a time when little or no change in stream temperature occurred. Furthermore later in the migration they entered the stream earlier in the evening which again seemed to be related to earlier onset of dusk rather than to changes in temperature. Other factors such as pH and dissolved gas content of the water considered important in regulation of diel timing of upstream sockeye migration by Krogius (1954) were not measured.

There may be strong selection for kokanee and other autumn spawning fishes which often must enter clear streams over shallow sills to do so during dusk or night, thus minimizing chances of capture by aquatic, aerial, and terrestrial predators. Munro and Balmain (1956) noted that brown trout migrating in late autumn into a spawning stream entered throughout the diel cycle when the water was high and turbid but restricted entry to night when

the level was dropping and the water clearing. River-spawning lake trout entered the Montreal River from Lake Superior shortly after dusk and reached a diel peak in this movement before midnight (Loftus, 1958).

Although the marked diel periodicity of stream entry in spawning kokanee may reduce predation, the mechanism of stream location, and the cause of the large daily fluctuations in numbers entering the stream, require more investigation. The preliminary experiments on nasal occlusion of kokanee, while inconclusive, suggested that olfaction may play a major part in the mechanism whereby these fish locate a small stream entering a relatively large lake. How a kokanee maturing at the southwest end of Nicola Lake might locate Moore Creek is not known. If olfaction is used by kokanee for stream location once they reach the northeast end of the lake, then a hypothesis can be developed to account for marked changes from day to day in the number of kokanee entering Moore Creek.

Mature kokanee moved onshore earlier in the evening and in greater numbers on afternoons when there were strong onshore winds. Furthermore large numbers of kokanee entered the creek on evenings when strong onshore winds were blowing while small numbers were associated with days of light onshore or offshore winds. It is suggested that this aspect of the migratory behaviour of kokanee is related to the effects of wind on the spatial distribution of Moore Creek water once it entered the lake. On calm days the creek water moved out in a narrow band across the sandy delta and dropped down the delta face along the bottom, whereas on days with strong onshore wind its influence could not be detected *thermally* more than a few meters laterally from its mouth. However, the creek water was undoubtedly mixed rapidly with lake water by heavy wave action and spread laterally along the shoreline. Although highly diluted, the creek might then have been detectable over a broad front by fish using olfactory cues which have been shown effective at remarkably low dilutions (Walker and Hasler, 1949). That olfaction was of importance in location of the creek used for spawning has been suggested in our nasal occlusion experiments as well as those on migrating coho salmon by Wisby and Hasler (1954).

If the chemical identity of the creek is broadcast along a greater area of shoreline on days with strong onshore wind, and if kokanee are using "creek odour" as a major cue in location of its mouth, then they should be able to find it more readily on such days than on days with only light onshore or offshore winds. Furthermore, kokanee appear to move onshore in greater abundance and earlier in the afternoon on days with strong onshore winds. Early onshore movement of kokanee may be brought about by reduction in light penetration caused by wind action since timing of both onshore movement and stream entry appeared to be related to light intensity (Fig. 3, 5, and 6). These three factors, (1) wider dispersion of "creek odour" along the shore, (2) greater numbers of kokanee in onshore areas, and (3) longer time spent by kokanee in onshore areas, may combine to account for the strong correlation between numbers of spawners entering the stream and wind velocity (Fig. 7).

Hayes (1953) and Huntsman (1939) both noted that onshore winds increased the number of Atlantic salmon entering spawning streams although in the majority of their observations the wind was accompanied by rain which increased stream discharge. In two cases, however, Hayes (1953) was able to observe the effect of wind alone on salmon migration and noted that large numbers of fish entered the stream on those occasions. The hypothesis put forth by Mottley (1938) relating the interaction of wind-driven lake currents, the stream current, and temperature in Paul Lake to numbers of rainbow trout spawners entering Paul Creek does not appear applicable to kokanee spawners entering Moore Creek as the low autumn discharge is rapidly dissipated horizontally by the strong onshore winds.

Movement of kokanee up Moore Creek itself took place largely during darkness and showed no tendency for evening and dawn "peaks" as shown for adult sockeye in the Dalnee River (Krogius, 1954). As in the latter river, upstream movement of kokanee in Moore Creek extended over a longer daily period when large numbers of fish were migrating. The importance of changes in pH and dissolved gas (O_2, CO_2) in controlling the diel cycle of upstream movement as suggested by Krogius (1954) could not be assessed. Temperature changes did not appear to be directly involved.

No well-grounded explanation can be given for the marked increase in length of kokanee spawning in Moore Creek between 1958 and 1962. Since the majority of kokanee growth probably occurs during the spring and summer of their first and second years in the lake and since the marked increase in size of mature *adults* occurred between 1959 and 1960, the causative factors must be looked for especially between 1958 and 1960. Although there are annual differences in temperature and oxygen conditions in Nicola Lake (Table I), these do not readily suggest themselves as direct causes for increased kokanee growth rate since 1959. Light penetration was lower in the summers of 1958 and 1959 than on subsequent years, largely because of heavy blue-green algal blooms, however, the differences do not seem particularly significant. Midsummer abundance of zooplankton was considerably greater in 1959 than in 1958 and remained so in 1961. Kokanee in Nicola Lake feed heavily on zooplankters at all times of the year, and indeed almost exclusively on them in autumn and spring (Lorz, MS, 1962). Increased size of mature kokanee in the lake since 1959 may be due to an increased abundance of zooplankton.

Rapid and large changes in size of mature kokanee have been noted elsewhere, perhaps most extensively in Lake Shikotsu by Mihara and Eguchi (1955), where fluctuations in plankton abundance brought about by artificial fertilization were considered causal. Johnson (1961) has described the interrelationship between growth rate of juvenile sockeye, their population density, and the abundance of zooplankton in Babine Lake, pointing out the direct dependence of growth rate on zooplankton abundance. Intraspecific competition was not felt to depress growth rates until young sockeye densities exceeded about 5000 fish per hectare. Unfortunately we have no measure of kokanee densities in Nicola Lake, however, judging from nearly complete enumeration

of spawners entering Moore Creek in 1958 and 1959 (approximately 5000 and 14,000, respectively) there may have been quite different densities of young in the two year-classes. Such differences, if present, apparently had little effect on the final size attained by the adults since spawners were about the same length in 1958 and 1959.

ACKNOWLEDGMENTS

The suggestions, criticisms, and extensive assistance given in the field by Dr G.F. Hartman are sincerely appreciated. Mr Gerard Guichon kindly permitted operation of the field camp and traps on his property. Conservation Officers A.F. Gill, E. Martin, and M.W. Reheis gave considerable assistance in the field as did Dr M.Y. Ali, Mr J.W. Cartwright, Dr J.G. Eales, Messrs C.A. Gill, T.G. Halsey, R.D. Humphreys, J.C. MacLeod, Dr T. Miura, Messrs J.S. Nelson, R.J. Paterson, D.C. Sinclair, C.E. Stenton, M. Teraguchi, K. Vermeer, and E.R. Zyblut. The criticisms of Miss J. Goodman, Dr W.S. Hoar, Dr C.C. Lindsey, Messrs J.S. Nelson, S.B. Smith, E.H. Vernon, and Dr F.J. Ward were most helpful.

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First Transponding Oceanographic Buoys in the Pacific[1]

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ABSTRACT

Three transponding oceanographic buoys were released off the Washington coast during May 1964. One drifted over 320 miles 155° T at a speed of almost 5 miles per day. Two buoys, 1000 yards apart on May 29, were recovered 58 miles apart on July 17. Attempts to track the buoys at 10-day intervals by aircraft were unsuccessful. Communications using a frequency of 2690 kc were unsatisfactory at ranges in excess of 200 miles, but an interrogation and transponding range of 900 miles was obtained using 4134.5 kc.

INTRODUCTION

Fishery research at this Laboratory has undergone significant changes in the last decade. Prior to 1953, priority was given to research associated with management problems in the Columbia River and Alaskan streams; a marine program was virtually nonexistent. From 1953 to the present time, a large part of the Laboratory's research has been directed toward answering problems confronting the United States Section of the International North Pacific Fisheries Commission. Most of these studies have been of a marine nature and have been carried out in the north-central North Pacific Ocean and Bering Sea (Dodimead *et al.*, 1963). Increased fishing activity of foreign vessels in the Gulf of Alaska has made it mandatory for us to know as much as possible about local fish stocks and the oceanographic conditions that affect their abundance and distribution, if they are to be protected by international agreements.

Movement of surface water off the west coast of the United States is very complex and not too well known. Drift bottle studies off the southern California coast indicate a northward flow of water along Oregon and Washington during winter (Reid, 1959), and there is good evidence of a southward flow during spring and summer (Budinger *et al.*, 1964). However, the predominant surface movement of oceanic water as indicated by drift bottles (Favorite, 1964) is onshore (Fig. 1). This condition is rather unique along the United States coastline and makes our area ideally suited for investigations using drifting buoys.

The purpose of our first experiment was threefold: to test the seaworthiness of radio buoys in the rough seas common to northern Pacific waters; to ascertain the range of transmission and reliability of the radio equipment; and to determine the feasibility of locating the buoys by ship and aircraft.

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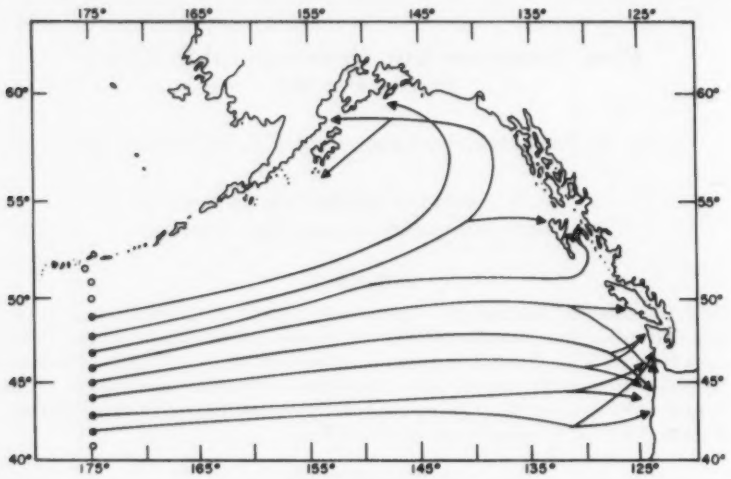

FIG. 1. Locations of release and recovery of drift bottles, 1962–64 (open circles indicate no recoveries).

DESCRIPTION OF BUOY

In order that our investigations be of maximum use to fishing interests, we felt that the information obtained should be instantly available and reflect existing rather than average conditions. Thus, we decided to use radio buoys that would measure oceanographic conditions and transmit data directly to our Seattle Laboratory. Use of batteries required that the buoys be transponding, transmit only when interrogated, so that power would be expended only when information was requested.

The buoy selected was manufactured by Concord Control, Inc., of Boston, Massachusetts. The case, 14 ft long and 8 inches in diameter, is constructed of aluminum tubing protected by a plastic coating and contains the battery and electronics assembly (Fig. 2). The lower end is closed by a damping plate and provides a RF sea ground, and a 13-ft antenna is attached to the top. A cylindrical plastic chamber, 3½ ft in diameter and 3½ ft high, filled with polyurethane foam, surrounds the top of the case providing stability and buoyancy, as well as providing protection for small craft. Large, alternating, horizontal fluorescent red and yellow bands permit identification of the buoy at distances of several miles. Each buoy, upon interrogation by a sequential two-tone radio signal, responds by transmitting its individual call sign and whatever intelligence is programmed into the electronics assembly (Morin and Walden, 1963).

RESULTS

Reconnaissance flights were conducted by U.S. Coast Guard aircraft from Port Angeles Air Station, and the buoys were released and recovered by the Bureau of Commercial Fisheries research vessel *George B. Kelez*. Several re-

FIG. 2. Telemetry buoy being hoisted aboard the RV *George B. Kelez* (13-ft whip antenna not visible).

strictions were imposed by available equipment and operating schedules. Although a frequency of 4134.5 kc had been selected for this experiment on the basis of considerations of maximum range of transmission with minimum of power loss, maximum bearing accuracy, and frequencies available for oceanographic use, the aircraft direction finding (DF) equipment was limited to only low or very high frequencies. The only frequency available that was compatible with both plane and buoy equipment was the Coast Guard frequency, 2690 kc, and we were given permission to use it during May, June, and July. Further, during the early phase of the experiment only one tone

generator was available to interrogate the buoys and it was installed aboard the *Kelez*. Finally, the initial release location and the number of buoys released were chosen with the realization that for this experiment the operating range of the aircraft was 300 miles, and that the services of the *Kelez* would not be available during June.

Our first experiment was conducted with two buoys tuned to 2690 kc. After initial tests with an aircraft in the Strait of Juan de Fuca on May 11, 1964, we released buoy KG7701 approximately 300 miles offshore at 47°30′N lat, 132°00′W long, and buoy KG7707 30 miles north at 48°00′N lat, 132°00′W long (Fig. 3). We had hoped that frequent positioning of the buoys would permit determining the nature of rotary currents known to be present offshore. During the morning of May 13, the buoys were interrogated by the *Kelez* and easily located by a Coast Guard aircraft following the DF bearing of the transponding signals. A maximum range of detection of about 200 miles, considerably less than we had estimated, and a bearing drift to the left as the buoys were approached, were reported.

During this phase of the experiment, we were not able to detect the buoy transmissions at the Bureau of Commercial Fisheries Exploratory Fishing Base radio station KAB, in Seattle. We believed, however, that when the *Kelez* returned to port and the tone generator was installed at KAB, sufficient power, 150 w, would be available to interrogate the buoys, and the 200-mile range of detection of the transponding signal from the buoys by aircraft would insure successful tracking. This was not the case. On May 20, during a flight over the estimated position of the buoys, no transmission from either buoy was detected following interrogations from KAB. On May 29, while the *Kelez* was en route to the Aleutians on another assignment, no difficulty was experienced in locating buoy KG7707 from DF bearings of its transmission in

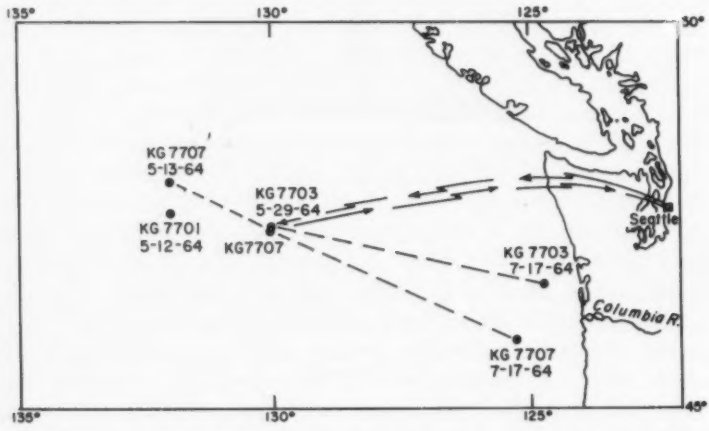

FIG. 3. Locations and dates of release and recovery of telemetry buoys, May–July 1964.

response to shipboard interrogations. It was found at 47°21′N lat, 130°00′W long, and had drifted approximately 90 miles 115° T. Visual inspection, without removing the buoy from the water, indicated that it was in excellent condition. At this time, we had tone generators aboard the *Kelez* and at KAB, and were able to determine that interrogations from KAB were received and responded to only at night. We decided to test communications using our assigned frequency, and on the same day, buoy KG7703, tuned to 4134.5 kc, was released ½ mile away from buoy KG7707. Communications were excellent and we were able to interrogate and receive the transponding transmission during day or night from KAB. No contact was made with buoy KG7701 and its fate is not known at this time. After a thorough search, the *Kelez* continued on to the Aleutians and maintained communication with buoy KG7703 for a distance of over 900 miles.

Through KAB, we were able to maintain communication with buoy KG7703 during June and July. Several other attempts were made to position the buoys from aircraft, but they were unsuccessful even though on one flight the tone generator was mounted in the aircraft.

Buoy KG7707 was recovered by the *Kelez* on July 17, 1964, at 45°54′N lat, 125°18′W long, after drifting over 320 miles at a speed of almost 5 miles per day. The three known positions of the buoy lie on a straight line, 115° T. Buoy KG7703 was recovered the same day at 46°42′N lat, 124°46′W long, approximately 58 miles away. Both were in excellent condition throughout.

CONCLUSIONS

We consider the experiment to have been a success and will continue to utilize radio buoys in our studies of oceanographic conditions off the Washington coast. We hope to add temperature sensors to the buoys and to seek ways for providing an aircraft DF capability at 4134.5 kc so that the actual paths of the buoys can be ascertained.

Although only three positions of buoy KG7707 are known, a general onshore movement is indicated. Results of a drift bottle study conducted during this period (Fig. 4) suggest that at the time of recovery the buoy had entered the coastal regime, in which the flow was southward.

Today, networks of moored oceanographic buoys have been proposed for recording meteorological and oceanographic data in the North Pacific Ocean. Because such networks will provide insight into fundamental questions of air–sea interactions basic to the production of weather across the United States, it is likely that these networks will become realities in the near future. Optimum use of data from these moored buoys in predicting favorable or unfavorable environmental condition for local fisheries will depend upon careful selection of buoy sites and knowledge of factors influencing movements of fish. As a part of the Bureau of Commercial Fisheries long-range plan, TRIDENT, our research will seek answers to these problems.

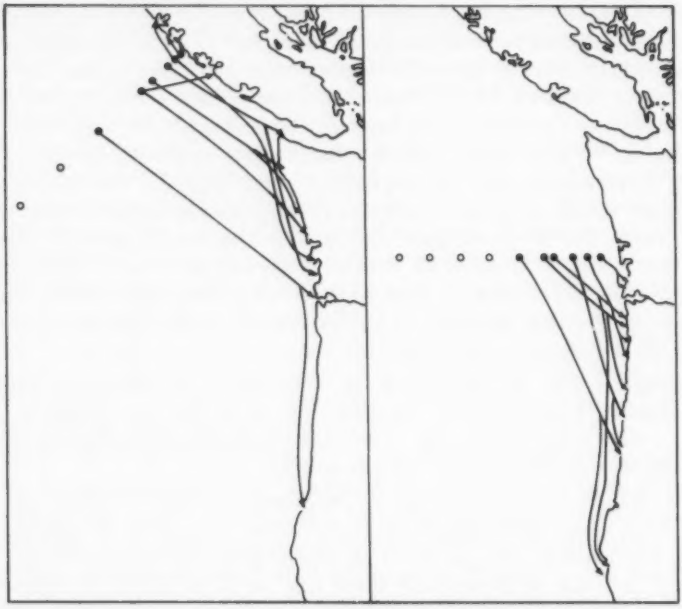

FIG. 4. Locations of release and recovery of drift bottles May–July 1964 (open circles indicate no recoveries).

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Sex Ratios and Sexual Dimorphism Among Recently Transformed Sea Lampreys, *Petromyzon marinus* Linnaeus[1]

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ABSTRACT

The sex, length, and weight were determined of nearly all recently transformed sea lampreys migrating downstream in the Carp Lake River, Michigan, in the fall, winter, and spring of 1960–61. Similar data were collected from samples of an earlier run in the Carp Lake River and of runs in three other tributaries of Lakes Huron and Michigan. The sex ratio of the 1960–61 migrants in the Carp Lake River was 324 males:100 females. Sex ratios of migrants in the other runs varied from 77 to 86 males:100 females. The high proportion of males in the 1960–61 run in the Carp Lake River is attributed to the effective prevention of recruitment of sea lampreys in the river and transformation of the females at an earlier age than is characteristic of the males. A near equal sex ratio among recently transformed migrants is considered normal for the species. The sex composition of a run changed during the period of migration. The proportion of males among the migrants was greatest at the beginning of the run and declined steadily thereafter. The average size was smaller for males than for females. Differences in the mean lengths and weights of the sexes were statistically significant. The length–weight relation differed for the sexes and showed a slower rate of increase of weight with increase in length than is characteristic of other stages of the animals' life cycle. Seasonal changes in the length–weight relation had a trend toward lower weights among the migrants coming downstream in the later months of the run.

INTRODUCTION

Researches on the life history of the parasitic sea lamprey (*Petromyzon marinus*) in the upper Great Lakes and on methods for its control have included much attention to the spawning runs of adults into streams. Records have been kept of the total numbers and the sex and size of spawning migrants entering the Ocqueoc River, Presque Isle County, Michigan, nearly every year since 1947. Similar records, covering shorter spans of years, have been collected for many additional tributaries of Lakes Huron, Michigan, and Superior. Early reports of these data were made by Applegate (1950), Applegate and Smith (1951), Applegate *et al.* (1952), and Erkkila *et al.* (1956); much of the information for later years has not been published.

Pronounced changes in sex composition with changes in the abundance of spawning migrants in successive years were described in these reports.

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Printed in Canada.

Subsequently, Applegate (unpublished data) demonstrated that abundance and the ratio of males to females were positively correlated in Ocqueoc River spawning runs, i.e. the greater the number of mature migrants the larger was the proportion of males. Hardistry (1961a) observed the same correlation between abundance and sex ratio in spawning populations of the nonparasitic *Lampetra planeri* in the River Yeo in England. He showed further the similarity of this relationship in the two species with data on the Great Lakes sea lamprey provided him for this purpose.

Differences in the life cycle of *L. planeri* (nonparasitic as an adult) and the sea lamprey (parasitic as an adult) make difficult any speculation about the factor or factors regulating sex composition among populations of the mature individuals. It is possible that a common explanation of the relation between population density and sex ratio will not be found for the two species. Unquestionably, any search for causal factors will be aided by a knowledge of the sex composition of larval populations and, for the sea lamprey, of downstream runs of recently transformed individuals.

The only significant report of sex ratios among larval populations is that of Hardisty (1960a) for *L. planeri*, *L. fluviatilus*, and *P. marinus*. In a small sample of larval sea lampreys taken from the Ocqueoc River, Michigan, he found an approximately equal sex ratio (48.6% males). No comparable information has been available for populations of sea lampreys immediately following their metamorphosis. Applegate (1950, 1961) and Applegate and Brynildson (1952) described the downstream runs of recently transformed sea lampreys in the Carp Lake River, Michigan, but they did not determine the sex of the individual migrants.

This report describes the sex composition of an entire downstream run of recently transformed sea lampreys in the Carp Lake River and of samples of migrating or sedentary populations of the same life-history stage in this and in three other Great Lakes tributaries. It also describes sex differences among downstream migrants in organ development, morphometry, and time of migration.

MATERIALS AND METHODS

Collection of Specimens

An inclined-screen trap for the capture of downstream-migrating sea lampreys and fishes was operated almost continuously in the Carp Lake River, a tributary of the Straits of Mackinac in Emmet County, Michigan, from April 1948 until September 1961 (Applegate and Brynildson, 1952; Applegate, 1961). During the fall, winter, and spring of 1960–61, a downstream run of 8925 recently transformed sea lampreys was taken in this device. Few, if any, migrants are believed to have escaped the trap during this season (Thomas M. Stauffer, Michigan Department of Conservation, personal communication). Of the total number of lampreys captured, 8793 or 98.5% of the run were available for this study. Samples of an earlier downstream run, captured in this river in 1956–57, were also at hand. These consisted of 370 migrants

collected at random in November and December 1956 and April and May 1957 from a total run of at least 2640 lampreys (Applegate, 1961).

Samples of populations of recently transformed sea lampreys in three other widely separated tributaries of Lakes Huron and Michigan were taken by several means. The largest collection consisted of 1862 downstream migrants of the 1962–63 run in the Pere Marquette River, a large stream flowing into Lake Michigan on its east-central shore in Mason County, Michigan. These lampreys were captured in a trap set in the water intake of a Dow Chemical Company plant about ¼ mile from the mouth of the river along the shore of Pere Marquette Lake. Of the total sample, 1235 specimens were caught between November 11 and 24, 1962, and 627 between February 11 and April 15, 1963.

Fyke nets took 408 downstream migrants of the 1962–63 run in the Ocqueoc River, a tributary of northern Lake Huron in Presque Isle County, Michigan. Of these lampreys, 119 were caught between December 3 and 10, 1962, and 289 between March 11 and April 10, 1963.

Transformed and partly transformed sea lampreys collected just prior to their downstream migration while they were still sedentary in the stream bed were obtained from the Ogontz River, Delta County, Michigan. This small stream, which enters Lake Michigan on its northwest shore, was treated with a selective lamprey larvicide on August 30–September 1, 1960 (Smith and McLain, 1962). Of the 576 transformed or partly transformed individuals picked up at random throughout the river system during the treatment, we examined 527.

With one exception, all lampreys were fixed in 10% formalin at the time of collection. Eighty-seven of the specimens taken in the Carp Lake River in 1956–57 were fixed in Bouin's solution and then transferred to 70% alcohol.

Determination of Sex

Sex differences are known to exist in the development of the reproductive organs of lampreys. Unfortunately, specific events and the time of their occurrence in the development of the gonads have not been defined clearly for any species. Okkelberg (1921) showed that ammocoetes of *Entosphenus wilderi* (Gage)[2] passed through an indeterminate or indefinite sex phase during their first year or so of life. At this stage mitotic divisions of the germ cells took place. Later all individuals entered a phase characterized by some meiotic divisions resulting in the formation of oocytes. This process continued in specimens destined to become females until the full complement of oocytes was formed. The production of oocytes in males ceased, however, and the gonad remained in a resting state with spermatogonia as the only vital germ cells; the oocytes that had already formed either degenerated or remained rudimentary. Meiotic maturation divisions preliminary to the formation of spermatozoa did not take place until a few months before spawning. More recent studies of the ammocoetes of other species suggest that this course of events is fairly

[2]Now called *Lampetra lamottei* (LeSueur).

typical of all lampreys, i.e. the two sexes are at very different stages of sexual development at the time of metamorphosis (Hardisty, 1960a, b, 1961b; Zanandrea, 1957).

Hardisty (1960b) has reported that a definitive ovary or testis can be found in ammocoetes of the sea lamprey that have a total length over 90 mm. Our examination of the gonads of recently transformed sea lampreys proved that the sex of the individuals is readily distinguishable. We have found further that sex differences in gonadal development are sufficiently pronounced to permit rapid and accurate macroscopic identification.

Microscopic examinations verified the accuracy of macroscopic determination of sex. Specimens for microscopic study were fixed either in Bouin's solution or in 10% formalin. Blocks were cut from various locations along the central part of the body, dehydrated by standard methods, embedded in paraffin wax, softened in Mollifex (B.D.H.), and then sectioned at 10 μ on a rotary microtome. Sections were stained with haematoxylin and counterstained in eosin.

Under low magnification, the cross-section of the gonad in the recently transformed male sea lamprey is small and evenly shaped and has a fine structure (Fig. 1 and 2). That in the female, on the other hand, is large and horseshoe-shaped and usually projects to some extent around both sides of the alimentary canal (Fig. 3 and 4). The oocytes in the female gonad are large and are visible in most specimens even under a hand lens. Germ cells in the testis have not progressed beyond the spermatogonial stage and can be seen only under high magnification (Fig. 5).

To develop a macroscopic technique for determining the sex of the bulk of the lampreys discussed in this report, we initially cut numerous thick, transverse sections through the body cavity for examination with binocular microscopes and hand lenses. Subsequently, we found it necessary only to make a single transverse cut through the ventral half of the body at a point opposite the anterior end of the dorsal fin. The body was then folded back for examination of the viscera by eye alone or with low-power hand lenses. Females were identified by the large ovary, with its discernible oocytes, that appears as a relatively dark, gray-colored organ in both fresh and formalin-preserved specimens. Males were distinguished by the small testis that appears creamy-white and has a much lighter color than the ovary.

Length and Weight

Measurements of total length were made to the nearest millimeter on conventional fish-measuring boards. Weights were determined to the nearest 0.1 g on Ohaus triple-beam balances.

SEX COMPOSITION

Of the 8925 recently transformed downstream migrants taken in the Carp Lake River trap in 1960-61, the sex was determined for 8780 individuals

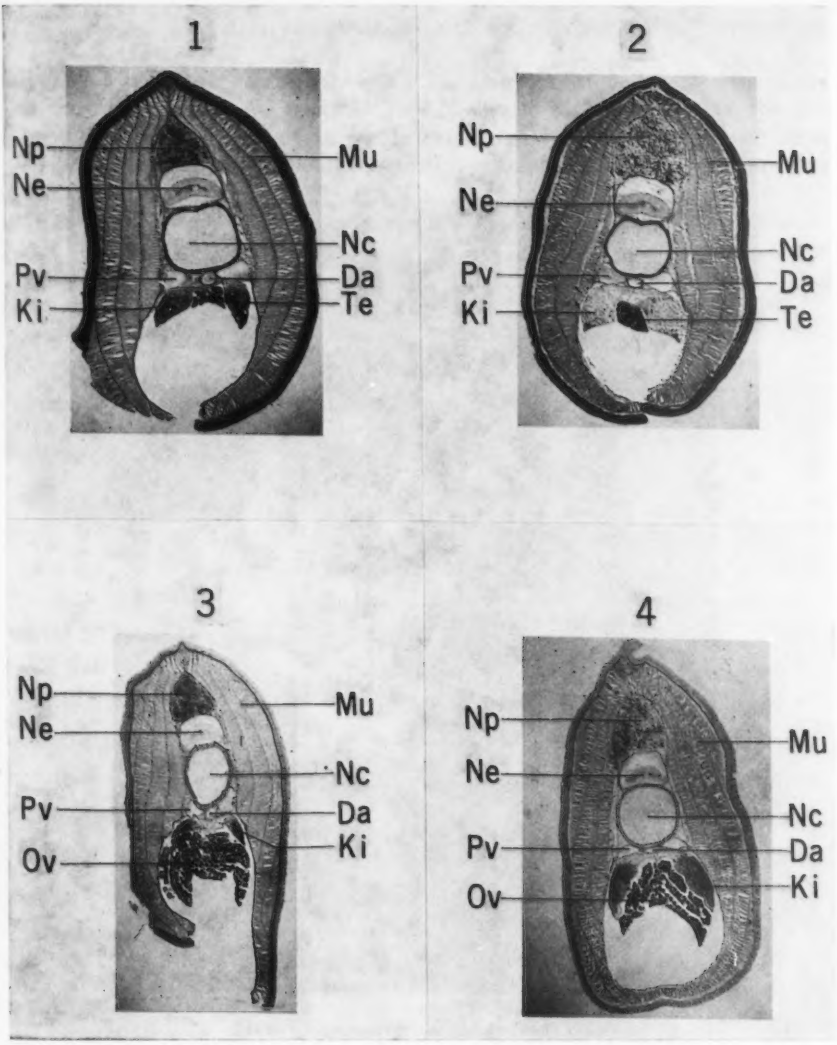

FIG. 1. Cross section of recently transformed male sea lamprey at anterior portion of body cavity (× 16). Alimentary canal removed before sectioning. Kidney in this body area is small and composed largely of nephric tubules. Da = dorsal artery; Ki = kidney; Mu = trunk musculature; Nc = notochord; Ne = neural cord; Np = neural process; Pv = post cardinal vein; Te = testis.

FIG. 2. Cross section of recently transformed male sea lamprey at posterior portion of body cavity. Alimentary canal removed before sectioning. Kidney in this area is large (lobed) and composed primarily of adipose tissue. Legend the same as in Fig. 1.

FIG. 3. Cross section of recently transformed female sea lamprey at anterior portion of body cavity. Alimentary canal removed before sectioning. Kidney in this body area is small and composed largely of nephric tubules. Ov = ovary; remainder of legend the same as in Fig.1.

FIG. 4. Cross section of recently transformed female sea lamprey at posterior portion of body cavity. Alimentary canal removed before sectioning. Kidney in this area is large (lobed) and composed primarily of adipose tissue. Ov = ovary; remainder of legend the same as in Fig. 1.

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FIG. 5. Section of the testis of a recently transformed male sea lamprey, showing spermatogonial cells (× 1200). Specimen was 144 mm, total length, and was captured moving downstream in the Ocqueoc River in March 1964.

or 98.4% of the total run. Of these 8780 lampreys, 6710 or 76.4% were males and 2070 or 23.6% were females. This is a ratio of 324 males:100 females (Table I). The sex composition of this run differed significantly from that of the run in the same river 5 years earlier. In a sample of 370 migrants captured in the Carp Lake River in 1956–57, 171 or 46.2% were males and 199 or 53.8% were females (ratio of 86 males:100 females — Table II). The proportion of males (or females) in samples of migrant and sedentary stocks of recently transformed sea lampreys collected in recent years in three other Great Lakes tributaries was similar to that for the Carp Lake River migrants in 1956–57. The sex ratio of the migrants of the 1962–63 run in the Pere Marquette River

TABLE I. Numbers of males and females and sex ratios among recently transformed sea lampreys migrating downstream in the Carp Lake River, Michigan, during semi-monthly periods in the fall, winter, and spring of 1960–61.

Semi-monthly period (1960–61)	Total number of migrants[a]	Number of males	Number of females	Sex ratio	
				Males: females	Percentage males
Sept. 15–30[c]	1	1	0	–	100.0
Oct. 1–15	12	12	0	–	100.0
16–31	721	584	137	426:100	81.0
Nov. 1–15	4050	3126	924	338:100	77.2
16–30	3049	2295	754	304:100	75.2
Dec. 1–15	346	263	83	317:100	76.0
16–31	62	45	17	265:100	72.6
Jan. 1–15	30	16	14	191:100[b]	65.6[b]
16–31	34	26	8		
Feb. 1–15	9	8	1	260:100[b]	72.2[b]
16–28	9	5	4		
Mar. 1–15	126	86	40	215:100	68.3
16–31	298	222	76	292:100	74.5
Apr. 1–15	30	19	11	175:100[b]	63.3[b]
16–30[c]	3	2	1		
Grand totals and averages	8780[a]	6710	2070	324:100	76.4

[a]Does not include 13 individuals for which sex was not determined.
[b]Data combined for monthly period because of small number of specimens.
[c]The first migrant was taken on Sept. 25, 1960; the last on April 28, 1961.

TABLE II. Numbers of males and females and sex ratio among samples of recently transformed sea lampreys collected in four Great Lakes tributaries.

Location and dates of collection	Total number of specimens	Number of males	Number of females	Sex ratio	
				Males:females	Percentage males
Carp Lake River					
Nov.–Dec. 1956	234	117	117	100:100	50.0
Mar.–Apr. 1957	136	54	82	66:100	39.7
Total sample: 1956–57	370	171	199	86:100	46.2
Pere Marquette River					
Nov. 11–24, 1962	1235	488	747	65:100	39.5
Feb. 14–Apr. 15, 1963	627	280	347	81:100	44.7
Total sample: 1962–63	1862[a]	768	1094	70:100	41.2
Ocqueoc River					
Dec. 3–10, 1962	119	54	65	83:100	45.4
Mar. 11–Apr. 10, 1963	289	120	169	71:100	41.5
Total sample: 1962–63	408[a]	174	234	74:100	42.6
Ogontz River					
Aug. 31–Sept. 1, 1960	527[b]	233	294	79:100	44.2

[a]Downstream migrants.
[b]Not migrant individuals; collection made during chemical treatment of stream with a selective lamprey larvicide.

was 70 males:100 females while that of the migrants in the Ocqueoc River in the same season was 74 males:100 females. The recently transformed but still sedentary sea lampreys taken from the Ogontz River in the early fall of 1960 had a sex ratio of 79 males:100 females (Table II).

We believe that the ratio of 324 males:100 females in the 1960–61 run in the Carp Lake River is atypical of the sex composition of recently transformed sea lamprey populations in Great Lakes streams. We attribute the high proportion of males in this run to (a) the effective prevention of recruitment to the larval populations in that stream for approximately 6 years immediately prior to the run and (b) to a heretofore unsuspected (and as yet unproven)

feature of the sea lamprey's life history, viz. that the females transform at an earlier age than do the males.

The dam and inclined-screen trap constructed near the mouth of the Carp Lake River in 1948 was intended not only to capture recently transformed downstream migrants but also to block the upstream spawning migration of mature sea lampreys and thus prevent subsequent additions to the larval populations of the stream. Unfortunately, some escapement of spawning migrants above the dam and consequent recruitment to the larval populations seems to have occurred at least through 1954 (Applegate, 1961; Stauffer, MS, 1956). According to Stauffer (1962), however, no recruitment occurred above the dam in 1955–61. On the basis of this conclusion, he stated further that the migrants of the 1960–61 downstream run for which we have reported the sex composition were at least 7 years old (1954 year-class). In the 5-year interval between the run of 1956–57 and that of 1960–61, the ratio of males to females in the downstream migrants increased nearly four-fold. In the absence of any recruitment for nearly 7 years, this pronounced shift in the sex composition of the runs can mean only that the females transformed and left the stream at an earlier age than did the males. This conclusion would be valid regardless of whether the 1960–61 downstream run was composed of one (1954?) or of several successive year-classes.

The ratio of 86 males:100 females in our sample of the 1956–57 downstream run in the Carp Lake River is unquestionably more nearly typical of populations of recently transformed sea lampreys since it agrees closely with the sex ratios in the collections from the Pere Marquette, Ogontz, and Ocqueoc Rivers where spawning and recruitment to the larval populations have been either largely unhampered or ineffectively prevented (Table II). The spawning runs of adult sea lampreys have been under some measure of control in the Pere Marquette and Ogontz Rivers in only 2 years. Electromechanical weirs and traps to block and capture the spawning-run migrants were operated in both rivers in 1958 and 1959. The supervisors of the operation of these two devices believe that some adults escaped to the spawning grounds in both years in both rivers. Either a mechanical or an electromechanical weir and trap for the capture and destruction of spawning migrants was operated in the Ocqueoc River in 10 of the 12 spring spawning seasons between 1949 and 1960. Some adults are known to have escaped this device and reached the spawning grounds in every year that the barrier was operated. Collections of larval lampreys made regularly in this river have never revealed complete elimination of recruitment in any year. We conclude from the similarity of the sex ratios in these four collections, made over a span of 7 years from widely separated streams, that populations of recently transformed sea lampreys in Great Lakes streams are normally characterized by a small but variable preponderance of females.

SEX DIFFERENCES IN TIME OF MIGRATION

The sex composition of the recently transformed sea lampreys of the Carp Lake River changed greatly during the migration in 1960–61. The proportion

of males among the migrants was greatest at the beginning of the run and declined irregularly but with a clear trend thereafter (Table I). The occasional individuals taken during the early weeks of the movement between September 25 and October 15, 1960, were all males. Females began to move downstream in mid October and their relative numbers increased until they comprised approximately one-third of the migrants captured in the last weeks of the migration in April 1961. Evidence has been presented previously that the great predominance of males in this run is not typical in populations of recently transformed sea lampreys. Consequently, the series of sex ratios given by us in Table I for successive intervals of the migration are not descriptive of the normal proportions of males and females. They do serve, nevertheless, to illustrate a differing trend in the time of downstream movement of the sexes.

Samples of two other runs give further indication of this sex difference in time of migration. The number of males and females was equal in the migrants collected in the Carp Lake River in November and December 1956. Another collection toward the end of this run in March and April 1957 included only 39.7% males (Table II). The downstream run of 1962–63 in the Ocqueoc River showed a similar change in sex ratio. The recently transformed sea lampreys captured in this river in December 1962 were 45.4% male. Among later migrants of March and April 1963, the percentage of males has declined to 41.5.

Samples of the 1962–63 run in the Pere Marquette River showed a change in sex composition opposite that in the other runs. Of the migrants taken in this river in November 1962, 39.5% were males, but a later collection, made during February, March, and April 1963, had 44.7% of males. We can offer no explanation for the differing trend in the time of migration of the males and females in this run.

LENGTH, WEIGHT, LENGTH–WEIGHT RELATION, AND SEASONAL DIFFERENCES IN SIZE OF DOWNSTREAM MIGRANTS

Length and Weight

The length and weight were determined for 8682 recently transformed sea lampreys (6630 males and 2052 females) from the Carp Lake River in 1960–61.

The males in this run were smaller than were the females. Total lengths of males ranged from 104 to 175 mm and averaged 145.0 mm (S.D. = ±8.3). Females ranged from 106 to 179 mm and averaged 148.0 mm (S.D. = ±8.2). The mean weight of the males was 4.6 g (S.D. = ±0.75); they varied from 1.6 to 7.7 g. Females averaged 4.9 g (S.D. = ±0.75) and ranged from 2.2 to 8.0 g. The frequency distributions of the lengths and weights of both males and females were all unimodal and highly symmetrical (Fig. 6 and 7). Characteristics of the length distribution for each sex agree closely with those reported for the combined males and females of downstream runs in earlier years in this river (Applegate and Brynildson, 1952; Applegate, 1961). Differences in the mean lengths and weights of the males and females were statistically significant. The standard error of the difference between the mean lengths was 0.2078;

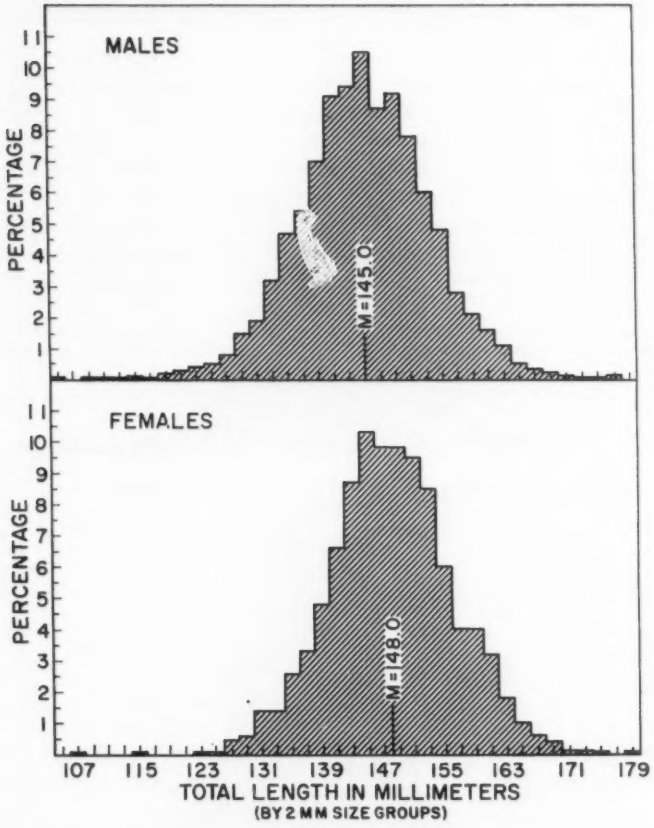

FIG. 6. Percentage frequency of lengths of 6630 recently transformed male and 2052 recently transformed female sea lampreys captured during their downstream migration in the Carp Lake River, Michigan, in 1960–61.

that of the difference between the mean weights was 0.0189. In both, the t ratios greatly exceed the 1% level of significance.

To explore further the sex difference in size of recently transformed sea lampreys, we also measured the lengths of 1862 migrants (768 males and 1094 females) collected in the Pere Marquette River in 1962–63. Again the mean length of the males was significantly smaller than that of the females. Total lengths of males in the samples of this run averaged 135.7 mm (S.D. = $\pm$9.8) and ranged from 109 to 170 mm. Females averaged 137.5 mm (S.D. = $\pm$9.4) and varied from 96 to 180 mm. The standard error of the difference between the mean lengths of the males and females was 0.453. The t ratio exceeded the 1% level of significance.

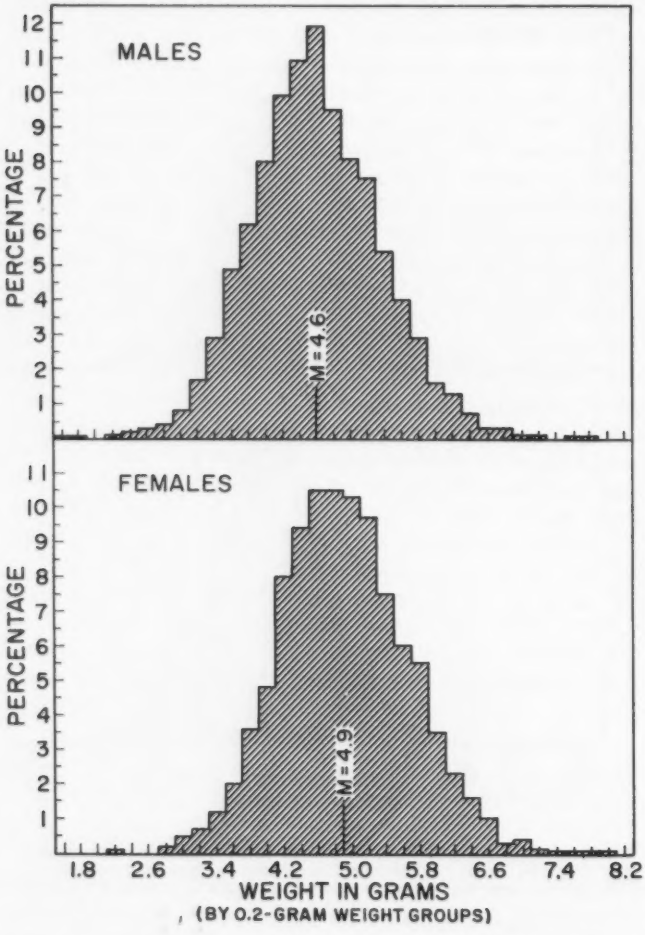

FIG. 7. Percentage frequency of weights of 6630 recently transformed male and 2052 recently transformed female sea lampreys captured during their downstream migration in the Carp Lake River, Michigan, in 1960–61.

Both male and female migrants in the Pere Marquette River averaged approximately 10 mm shorter than those in the Carp Lake River. Substantial variations in the growth of larval lampreys and in the average size of recently transformed downstream migrants in different streams have been reported to us by personnel engaged in sea lamprey control. We presume that these differences in larval growth and size at transformation are the result of varying population densities and suitability of larval habitat in different waters.

Seasonal Differences in Length and Weight

Applegate (1961) has shown that the size of recently transformed sea lampreys increases progressively during the period of downstream movement. The average total length of both male and female migrants of the 1960–61 downstream run in the Carp Lake River, computed for successive semi-monthly periods, was least in the autumn, increased during the early winter, and was greatest during the late winter and early spring (Table III). A similar, progressive increase in weight was lacking in the lampreys of this run. The average weight for periodic samples of male migrants was least in the early autumn, greatest in the late autumn and early winter, and less again in the late winter

Table III. Average lengths and weights of males and females among recently transformed sea lampreys migrating downstream in the Carp Lake River, Michigan, during semi-monthly periods in the fall, winter, and spring of 1960–61.

Semi-monthly period (1960–61)	Males			Females		
	Number measured	Average length	Average weight	Number measured	Average length	Average weight
		mm	*g*		*mm*	*g*
Sept. 15–30	1	(149)	(5.1)	0	–	–
Oct. 1–15	12	138.7	3.9	0	–	–
16–31	584	143.0	4.3	137	146.4	4.8
Nov. 1–15	3046	144.5	4.6	906	147.0	4.9
16–30	2295	144.5	4.6	754	147.9	4.9
Dec. 1–15	263	145.3	4.4	83	149.0	4.8
16–31	45	145.7	4.2	17	146.5	4.3
Jan. 1–15	16	145.8[a]	4.2[a]	14	149.5[a]	4.9[a]
16–31	26			8		
Feb. 1–15	8	143.2[a]	4.1[a]	1	156.0[a]	5.1[a]
16–28	5			4		
Mar. 1–15	86	147.8	4.5	40	147.9	4.7
16–31	222	145.2	4.2	76	147.8	4.5
Apr. 1–15	19	146.2[a]	4.4[a]	11	150.6[a]	5.0[a]
16–30	2			1		
Grand totals and averages	6630	145.0	4.6	2052	148.0	4.9

[a]Averages computed for monthly period because of small number of specimens.

and early spring. Average weights of female migrants varied from period to period but did not display a well-defined trend toward either an increase or a decrease during the run. Among the females captured during the periods of peak migration in November and March, however, the autumn migrants were heavier than those moving downstream in the spring.

It is evident from these data on length and weight that the "condition" of the recently transformed downstream migrants varies during the migratory period. Seasonal changes in "condition," as reflected in variations in the length–weight relation of the migrants, are described in a subsequent section.

Length–Weight Relation

The length–weight relation of recently transformed sea lampreys was calculated from the measurements of 8682 migrants from the 1960–61 downstream run in the Carp Lake River. The general length–weight equation, $W = cL^n$, where W = weight, L = length, and c and n are empirically determined constants, was fitted separately to data from 6630 males and 2052 females. All curves were fitted by least squares to mean lengths and weights for 2.0-mm intervals of length.

The logarithmic forms of the length–weight equations are:

$$\textit{Males} \quad \log W = -4.93791 + 2.585 \log L,$$

$$\textit{Females} \quad \log W = -4.44623 + 2.363 \log L,$$

where W = weight in grams, and L = total length in millimeters.

The empirical data for each length interval (including several intervals that were represented by only one or two lampreys) and the curves based on the above equations are shown in Fig. 8 (A) for the males and Fig. 8 (B) for the females. Most of the disagreement between empirical and calculated weights of both males and females was in the largest and smallest size groups which were represented by few specimens. Agreement between empirical and calculated weights of both sexes was generally very good despite differences in the length–weight relation among the lampreys coming downstream in different months of the migration period; these seasonal variations are discussed in the next section.

Comparison of the logarithmic equations describing the length–weight relation of the males and females indicates a difference between the sexes which is not readily apparent in the calculated curves of Fig. 8 (A) and (B). This difference is illustrated best by the logarithmic lines for the sexes as shown in Fig. 8 (C). The weight of recently transformed male sea lampreys increased at a greater rate with increasing length than did that of the females. In the smallest migrants, the females were significantly heavier, length for length, than the males. This sexual difference diminished as the length of the migrants increased and disappeared among the longest individuals. Differences in "form" and "fatness" between the sexes at this stage of the sea lampreys' life cycle,

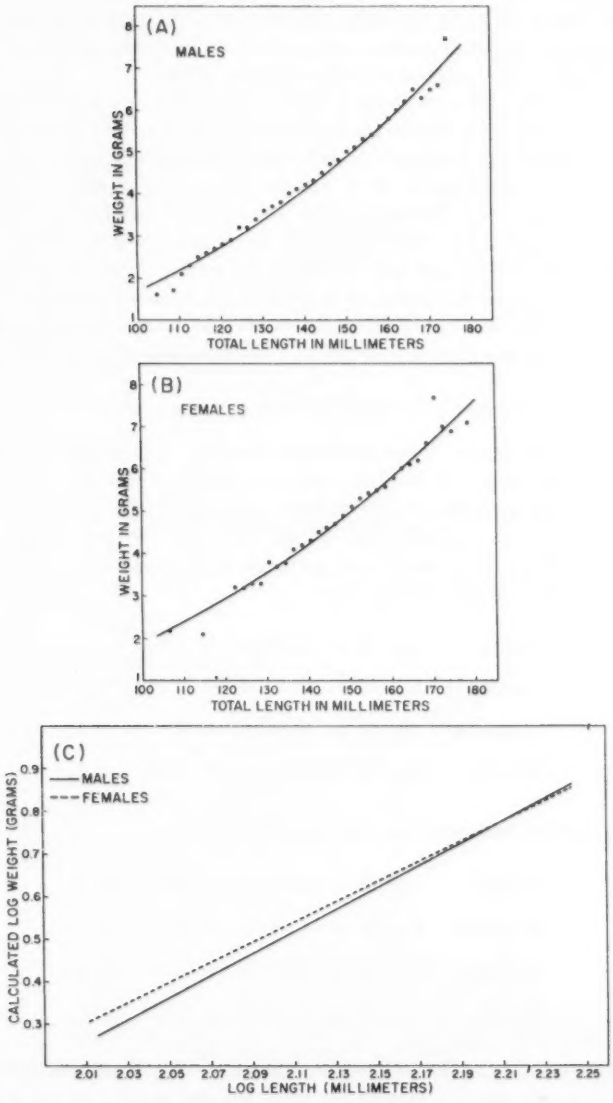

FIG. 8. Length–weight relation of recently transformed sea lampreys of the 1960–61 downstream run in the Carp Lake River. The curves of (A) and (B) represent the calculated weights and the dots, the empirical weights for each length interval. Graph (C) gives the relation for male and female sea lampreys in terms of calculated log weight and log length; empirical weights are omitted from (C).

though not extreme, are still of biological import. The greater weight of the females at nearly all sizes probably reflects an even greater weight advantage at the onset of metamorphosis; the considerable development of the ovary must have taken place at the expense of body tissue.

The mean value of n of about 2.47 for all of the Carp Lake River migrants is exceptionally low for a fish and lower than for other stages of the sea lampreys' life cycle. In adult sea lampreys of several successive spawning runs in a Lake Huron tributary, the value of n varied between 2.9 and 3.0 (Applegate, unpublished data). The length–weight relation of larval sea lampreys from tributaries of the Finger Lakes of New York has been described by Wigley (1959). Although Wigley did not calculate the equation $W = cL^n$, examination of his data shows that the value of n for the larvae would also be close to 3.0. The reason for the less rapid increase of weight with length in the recently transformed migrants than in earlier and later stages of their life is not clear.

Seasonal Differences in Length–Weight Relation

Sufficient specimens were collected during the peaks of downstream movement in October, November, and December 1960 and March 1961 to permit comparisons of the length–weight relation of the lampreys in each of these months. The logarithmic forms of the length–weight equation (W = weight in g; L = length in mm) that describe the relation in male migrants in each of these months are:

$$\textit{October} \quad \log W = -4.98697 + 2.605 \log L,$$

$$\textit{November} \quad \log W = -4.57705 + 2.424 \log L,$$

$$\textit{December} \quad \log W = -4.51058 + 2.383 \log L,$$

$$\textit{March} \quad \log W = -4.75917 + 2.489 \log L.$$

The corresponding equations for females are:

$$\textit{October} \quad \log W = -4.02751 + 2.175 \log L,$$

$$\textit{November} \quad \log W = -4.51073 + 2.395 \log L,$$

$$\textit{December} \quad \log W = -4.41501 + 2.341 \log L,$$

$$\textit{March} \quad \log W = -5.56958 + 2.871 \log L.$$

Graphs of the logarithmic lines are shown in Fig. 9 (A) for the males and Fig. 9 (B) for the females (empirical data on the weight at each length are omitted to simplify comparisons of the lines).

Month-to-month differences in the rate of increase of weight of male lampreys with increase in length and in the actual weight did occur but did not follow a consistent seasonal trend. Weight increased most rapidly in October, least rapidly in December, and at intermediate rates between these

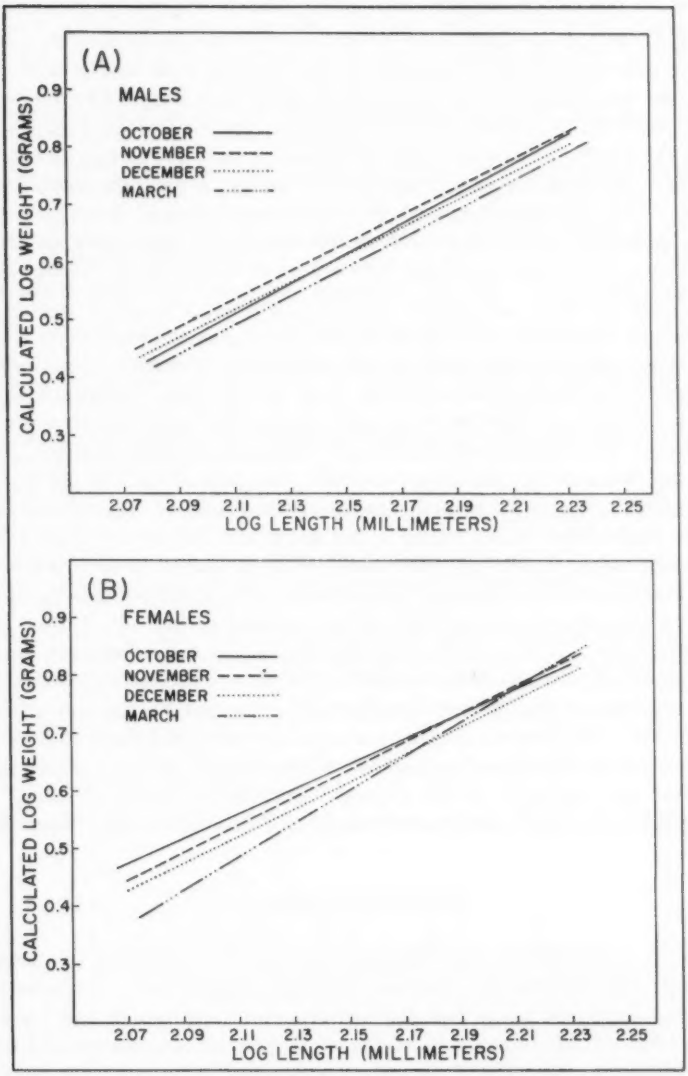

FIG. 9. Seasonal changes in the length–weight relation of recently transformed sea lampreys of the 1960–61 downstream run in the Carp Lake River. Data presented as calculated log of weight in grams and log of length in millimeters. Empirical weights and all length groups that included less than four individuals omitted from these graphs. (A), male sea lampreys; (B), female sea lampreys.

extremes in November and March. At equivalent lengths, the males were heaviest in November, lightest in March, and intermediate in October and December.

The rate of increase of weight of the females with length also changed during the run. Weight increased least rapidly with length in October, most rapidly in March, and at intermediate rates in November and December. Among the smaller individuals, the females were heaviest at equivalent lengths in October and progressively lighter in November, December, and March. As the size of the females increased this progressive loss of weight during the migratory period became less evident. Among the longest females there was little, if any, difference in weight between early migrants in October and late migrants in March.

Seasonal changes in the length–weight relation of the recently transformed downstream migrants can best be summarized as a general progressive loss of weight during this period in which they do not feed. A curious feature of the Carp Lake River migrants, however, was that the males coming downstream in November were significantly fatter than those migrating in October, although weight did decrease in subsequent months. Weights of females did not follow a similar trend. We cannot explain the increased weight of the males in November. We might understand better if we knew the history of these lampreys prior to migration. Metamorphosis usually starts in late summer and is complete by the time the lampreys migrate downstream. Individuals destined to transform in a given season, however, do not do so in a brief period nor do they migrate at the same time. It is possible that the November male migrants were from a local population of relatively heavy ammocoetes that had enjoyed a better-than-average food supply. They might also represent an aggregation of ammocoetes that continued to feed somewhat longer than did those that produced the lampreys moving downstream in October. Lower weights of migrants in the winter and spring is to be expected because of the lengthening period during which the transforming and transformed individuals have been without food.

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Oral Immunization of Rainbow Trout (*Salmo gairdneri*) Against an Etiologic Agent of "Redmouth Disease"[1]

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ABSTRACT

Rainbow trout were fed a pelleted diet containing killed cells of the etiologic agent of a bacterial disease, redmouth. These fish in addition to appropriate controls were subsequently challenged with virulent homologous organisms. Ninety per cent of the redmouth immunized fish survived the basic challenge using virulent organisms in contrast to 20% survival for the controls. Multiple challenge doses at increased levels also are discussed.

INTRODUCTION

Until recently little apparent interest has been shown in preventing infectious diseases in hatchery-reared fish through oral immunization. For the most part, control of the many infectious diseases in these fish has been through the administration of various bactericidal and bacteriostatic agents.

Duff (1942) demonstrated resistance to virulent *Aeromonas salmonicida* in 1- and 2-year-old cutthroat trout, *Salmo clarki*, that had received the homotypic chloroform-killed organisms in their diet over a prolonged period. The survival rate following introduction of the virulent organisms into the holding tank water was about 75% in the immunized group compared to about 25% in the control unimmunized group. In a subsequent challenge of other fish via intramuscular inoculation of a three-fold lethal dose to each, there was 32% survival in the immunized group versus 10% survival in the unimmunized control group.

Snieszko and Friddle (1949) were unable to demonstrate any protection in fingerling brook trout (*Salvelinus fontinalis*) against parenteral administration of heavy suspensions of virulent *A. salmonicida*. These fish had been exposed to homotypic heat-killed organisms in their diet.

Post (1963) found that rainbow trout (*Salmo gairdneri*) having received heat-killed *A. hydrophila* cells in their diet daily for 272 days were afforded questionable protection to subsequent challenge to homotypic virulent organisms. Following intraperitoneal inoculation of the challenge organism on four occasions, 183–418 days after the initial feeding of the bacterin, there was 25–28% survival in the immunized groups and 12.5% survival in the unimmunized control group.

Krantz *et al.* (1964) attempted to produce anti-*A. salmonicida* immunity in brook trout and brown trout (*Salmo trutta*) by feeding chloroform-killed

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homotypic organisms mixed in the diet. They concluded that their techniques were unsatisfactory to induce specific immunogenesis.

This report describes the induction of immunity in rainbow trout to the virulent etiological agent of redmouth disease. This immunity resulted from the administration of the homologous bacterin in a pelleted diet.

MATERIALS AND METHODS

Antigen

The organism used was a peritrichous, gram-negative bacillus that has been responsible for severe epizootics in rainbow trout hatcheries in Idaho for years. Attempts to taxonomically orient this organism have been unrewarding. About all that can be said in this regard is that it possibly belongs in the *Enterobacteriaceae*. It apparently is unrelated to *A. liquefaciens* (*A. hydrophila*), the classical etiological agent of redmouth disease in rainbow trout. A manuscript characterizing the bacterium is in preparation.

Three cultures of the test organism were isolated on Trypticase Soy Agar (TSA-BBL) from the kidney tissue of moribund rainbow trout. These fish were obtained from the National Fish Hatchery, Hagerman, Idaho, in the epizootic area. The three isolates were identified biochemically as the typical organism isolated from cases of redmouth disease. A portion of a pooled saline suspension of three isolates was inoculated intraperitoneally into several yearling rainbow trout. The resultant pure culture isolates from these fish were used to seed TSA plates. After 48 hr incubation at 72°F (22.2°C), the organisms were harvested and suspended in 0.5% aqueous phenol for 24–48 hr. They were then washed three times with sterile physiological saline. After testing for sterility, 15 ml of packed wet organisms were well mixed into 10 lb (4.54 kg) of commercial trout feed meal which was subsequently pelleted.

Fish Immunized

Three groups of 100 yearling rainbow trout were obtained from the National Fish Hatchery, Quilcene, Washington, where redmouth disease has not been discovered. During this study each group was maintained in 80-gal glass-walled aquaria supplied with 60 ± 2°F (15.5°C) dechlorinated Seattle, Washington, municipal water. The fish in each group to be challenged with virulent organisms were removed from the large aquaria and placed into 22.5-gal stainless steel tanks supplied from the above water source.

Preparation of Challenge Culture

The stock culture to be used was subjected to six passages in yearling rainbow trout to increase the virulence. Cultures of the isolates from these fish were lyophilized in rubber-stoppered glass vials and stored at −10°F (−27°C).

The basic challenge dose was based on the concentration of organisms in 0.2 ml saline that when injected intraperitoneally into yearling rainbow

trout produced death in 90% of the fish within 10 days after injection (LD_{90}). The concentration of the organisms was determined turbidometrically. Repeated tests verified the LD_{90} concentration.

After two challenges using the vials from the original culture, a marked decrease in virulence in this lot occurred as evidenced by having to increase the concentration to obtain a LD_{90}. This necessitated re-isolating the organism from a hatchery epizootic. After lyophilization of this isolate and then reconstituting it, the LD_{90} was found to be the same as with the original isolate. The virulence of this isolate remained stable throughout the rest of the study.

Feeding Schedule

One hundred yearling rainbow trout were fed the prepared pellets containing the bacterin. The initial feeding period was five times a week for 2 weeks. Subsequent feeding of the bacterin-containing pellets was at weekly intervals. Pellets without bacterin were fed to the test fish the other 4 days of the week after the initial period and were also fed throughout the study to the 100 or so control fish. Bacterin was not fed to any group of fish following the initial challenge.

Challenge Technique

Each challenge employed organisms reconstituted at the time of use from lyophilized material grown on TSA plates for 48 hr. The inoculum was prepared turbidometrically by harvesting these plates in the required amount of sterile saline based on the LD_{90} calculation. Each fish, test and control, received 0.2 ml intraperitoneally.

Preparation of *Aeromonas salmonicida* Bacterin

Pure cultures of *A. salmonicida* were isolated from fish naturally infected with the organism. The sequence of preparation of the bacterin followed that described for redmouth. The bacterin was then fed to 50 yearling rainbow trout following the same schedule as with redmouth.

Another group of 50 yearling rainbow trout received both the redmouth and *A. salmonicida* bacterins. This mixture was prepared by combining equal quantities of pellets containing each bacterin separately. This combination was fed to the fish in the same fashion as was described for the redmouth bacterin-containing pellets.

RESULTS

Seventy days after initiation of feeding the bacterin-containing pellets, ten fish fed the bacterin and ten fish on regular feed were inoculated intraperitoneally with 1 LD_{90} of the virulent stock culture. After 11 days there were nine survivors in the orally immunized group and one survivor in the nonimmunized control group (Table I). As each mortality occurred, material from the kidney was cultured on TSA plates and the homologous organisms

TABLE I. Summary of response of immunized and control rainbow trout to challenge doses of virulent redmouth organisms at several time intervals.

Days	Survivors: Immune	Survivors: Control	Dosage	
70	9/10	1/10	1 LD_{90}	
98	10/10	4/10	1 LD_{90}	
304	9/10	1/10	1 LD_{90}	
339	9/9	4/10	5 LD_{90}	— previous challenge: 304 days
	10/10	1/10	1 LD_{90}	
360	7/10[a]	2/10[b]	1 LD_{90}	
		1/10	1 LD_{90}	
	11/16	0/10	40 LD_{90}	
408	9/9	1/10	10 LD_{90}	— previous challenges: 304 and 339 days
	Total: 45/50 (90%)	12/60 (20%)	1 LD_{90}	
	9/9 (100%)	1/10 (10%)	10 LD_{90}	
	11/16 (69%)	0/10 (0%)	40 LD_{90}	

[a] These fish were fed a mixture of redmouth and *A. salmonicida* bacterin.
[b] These fish were fed *A. salmonicida* bacterin.

isolated. The two groups were observed for 5 weeks after being inoculated to check for delayed mortality. There was none.

On the 98th day, ten fish in the immunized group and ten fish in the control group were inoculated intraperitoneally with 1 LD_{90} of the virulent stock culture. Thirteen days later there were ten survivors in the immunized group and four survivors in the control group. As in the initial challenge, the homologous organisms were isolated from the kidneys of the fish that died.

The third challenge was done at 304 days. Three groups of ten fish each were inoculated intraperitoneally with 1 LD_{90} of virulent organism. One group was orally immunized, one was from the original unimmunized control stock, and one was from the same brood year as the rest but only recently introduced into the laboratory from the hatchery. This latter group was being used as a new negative control group and had no known previous contact with the organism in question. The original control group had been depleted by the repeated LD_{90} testing. Eight days after challenge, the number of survivors in each group was: nine in the immunized group; one in the original control group; and four in the new control group.

The nine survivors in the immunized group were re-challenged 35 days later (the 339th day) with 5 LD_{90} of the virulent organism and all fish survived. It should be noted that these fish received no bacterin-containing feed subsequent to the original challenge.

Also on the 339th day, ten immunized fish (not previously challenged) and ten controls were inoculated intraperitoneally with 1 LD_{90} of the virulent culture. Four days later there was one survivor in the control group, and there were no deaths in the immunized group. These survivors were observed for the following few weeks for any delayed mortality. There was none.

On the 360th day, five groups of fish were challenged. The first contained ten control fish; the second, ten fish that had received *A. salmonicida* bacterin, and the third, ten fish that had received both the *A. salmonicida* and redmouth bacterins. These received 1 LD_{90} of the stock redmouth culture intraperitoneally. The fourth group contained ten unimmunized controls and the fifth contained 16 fish from the redmouth immunized group; these received 40 LD_{90} of the redmouth culture intraperitoneally. Thirteen days later there was one surviving control fish that had received 1 LD_{90} and no survivors in the 40 LD_{90} controls. Two *A. salmonicida* immunized fish and seven of the *A. salmonicida*-redmouth immune fish survived the 1 LD_{90} inoculation. There were 11 out of 16 survivors in the redmouth-immune group that had received 40 LD_{90}.

The final challenge in this series was done at 408 days. The immunized group was comprised of the nine fish that had survived 1 LD_{90} at 304 days and a second challenge of 5 LD_{90} at 339 days. These fish had been on regular pelleted feed since the 304th day. There were ten unimmunized fish in the control group. Both groups received 10 LD_{90} intraperitoneally. Three days later there were still nine fish in the test group and only one in the control group. No further mortalities occurred following several weeks of observation.

DISCUSSION

Although many questions are left unanswered, preliminary studies in our laboratory indicate that oral immunization against redmouth disease may be an efficient method of controlling losses in production hatcheries.

More work is required to determine when resistance is effected and for how long a period of time the fish can be expected to be refractory following withdrawal of the bacterin-containing food. As the level of antigen was selected arbitrarily, it is very possible that other concentrations may be more satisfactory.

It would appear from the data that rainbow trout can be immunized against redmouth disease in a shorter feeding period since good protection was obtained in the initial 70-day challenge. Also, some protection was noted in the fish immunized against both the redmouth organism and *A. salmonicida* although the homologous antigen was only 50% of the level used in the other experiments. Furthermore, it is rather unlikely that fish in a hatchery environment would be subjected to as massive a dose of viable organisms at any one time as was used in these experiments.

It is difficult to assess the length of time that immunity may be maintained in the host following withdrawal of the antigen on the basis of the nine fish receiving the three series of challenges. Although these fish survived 10 LD_{90} 105 days after they were taken off food containing bacterin, it is probable that the two preceding challenges further enhanced the immune response.

The sample size of ten fish immunized against *A. salmonicida* and challenged with redmouth organisms is too small to be of statistical significance; however, there is no suggestion that cross immunity is conferred by the strain of *A. salmonicida* used.

Circulating antibody titers were not determined in this preliminary study since the correlation between *in vivo* and *in vitro* methods is highly variable (Cushing and Campbell, 1957). However, specific circulating agglutinins were detected in the undiluted serum from several of the test fish before they had been challenged with the virulent organism. The serum from corresponding control fish had no detectable circulating agglutinins for redmouth organisms.

Many infectious diseases of hatchery-reared fish can be controlled to some extent through use of chemotherapeutic agents; however, this approach is not without problems. In many instances the drug or antibiotic is bacteriostatic rather than bactericidal, and the disease, after regressing, once again becomes manifest following withdrawal of the treatment. Coupled with this is the potential emergence of resistant forms of the bacteria which subsequently do not respond to either the initial or related chemotherapeutic agents. It has also been noted that required concentrations of certain therapeutic agents render the food unpalatable to the fish, and consequently the medicated food is rejected. The bacterin-containing feed appeared entirely palatable. Thus a number of advantages are to be gained from a successful immunization program.

Immunization of trout can be effected by two routes, oral and parenteral, and in most cases, the parenteral route is more efficacious (Krantz *et al.*, 1964). It is not necessary, however, to elaborate on the disadvantages of injecting a bacterin into hundreds of thousands of young fish as might be required at a production hatchery. It thus becomes obvious that an oral type vaccine would be most feasible under hatchery production conditions.

At the time of writing, a hatchery trial involving a large number of rainbow trout has been in progress for over 6 months. The results obtained so far are very rewarding in terms of the numbers of fish surviving an experimental epizootic of redmouth. The data is to be presented in a subsequent paper.

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Effects of Fish Introductions and Hydroelectric Development on Fishes in the Kananaskis River System, Alberta[1,2]

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ABSTRACT

Changes occurred in the abundance and distribution of fishes in the Kananaskis River system, Alberta, in conjunction with fish introductions and hydroelectric development. Data from surveys from 1936 to 1961 indicate the probable chronology of events.

Dolly Varden (*Salvelinus malma*), brook trout (*S. fontinalis*), cutthroat trout (*Salmo clarkii*), and rainbow trout (*S. gairdneri*) decreased in abundance, probably due to the introduction of brown trout (*Salmo trutta*), longnose suckers (*Catostomus catostomus*), and white suckers (*C. commersonii*), to the cooling of the Kananaskis River from reservoir construction, and to sport fishing. Hybridization between rainbow and cutthroat trout was also important in the decrease of the latter species. After introduction by man, brown trout, rainbow trout, longnose suckers, white suckers, lake chub (*Hybopsis plumbea*), and longnose dace (*Rhinichthys cataractae*) greatly increased in abundance. Prior to the increase in numbers of white suckers, a reduction in the numbers of longnose suckers occurred in Lower Kananaskis Reservoir. Little change in the distribution of mountain whitefish (*Prosopium williamsoni*), longnose dace, and brook sticklebacks (*Culaea* (= *Eucalia*) *inconstans*) occurred over the 25 years. Changes in the physicochemical environment and invertebrate fauna in the reservoirs appeared to be of secondary importance to the interaction among fish in causing the changes in species abundance and distribution.

INTRODUCTION

With man changing the environment at an increasing rate, it has become important to predict the effects of proposed activities and to establish causes of undesirable effects associated with past activities upon abundance and distribution of fishes. Most studies on impounded waters lack adequate information on pre-impoundment conditions. Many reports exist on proposed impoundments with subsequent investigations not being conducted or the reservoir not being constructed. Predictions frequently enter these reports with little supporting data and no follow-up study. A paucity of information also usually exists on waters into which fish have been introduced.

Studies on the Kananaskis River system, Alberta, have been conducted in various stages of fish introductions and construction of four reservoirs starting in 1936. It is the aim of this paper, using past studies together with new information, to assess the changes in the fishes and attempt to determine causes.

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MATERIALS AND METHODS

Previous studies on fishes in the Kananaskis River system (Fig. 1) were used to establish chronology of events. Kananaskis Reservoir was examined by D. S. Rawson in 1938 and 1947 (Rawson, MS, 1939a, MS, 1948). Barrier Reservoir was examined by Rawson in 1947, the year it was filled; by J. R. Nursall in 1948, 1949, and 1960; and by D. J. B. Fillion from 1960 to 1963 (Rawson, MS, 1948; Nursall, MS, 1949, 1952, MS, 1960; Fillion, MS, 1963). Lower Kananaskis Lake was examined by Rawson in 1936 and 1937 (Rawson, MS, 1937, MS, 1948), and by R. B. Miller in 1954 (Miller, MS, 1954) during dam construction. R. C. Thomas made a post-impoundment study in 1957, and Fillion in 1961 and 1962 (Thomas, MS, 1957; Fillion, MS, 1963). Upper Kananaskis Reservoir was examined by Rawson in 1936 and 1947, and by Fillion in 1962 and 1963 (Rawson, MS, 1937, MS, 1948; Fillion, MS, 1963).

New data were collected, May 8 to September 8 and October 26–28, 1961, from Kananaskis Reservoir, south along Kananaskis River, to tributaries of Upper Kananaskis Reservoir (Fig. 1). Limnological measurements were made weekly at two stations located near the outlet and inlet of Barrier Reservoir and monthly on Lower and Upper Kananaskis Reservoirs. Temperature series were measured with a Whitney Underwater Thermometer. Dissolved oxygen was determined by the method of Miller (1914). A Hellige comparator with phenol red-D was used to measure pH. A Secchi disk gave an index of light penetration. The temperatures of the Kananaskis River, various tributaries, and beaver ponds were measured with a Taylor hand thermometer.

Fish were collected with gillnets, seines, dipnets, and rotenone. Monofilament nylon gillnets of 1–4 inches (25.4–101.6 mm) stretched mesh were set 3 times in Kananaskis, 67 in Barrier, 5 in Lower Kananaskis, and 4 in Upper Kananaskis Reservoirs. Nets were fished at several stations at various depths and times of day throughout the season in Barrier Reservoir. Nets were set in several localities within each reservoir in this and past surveys. This, plus the relatively uniform distribution of the species around the shoreline makes it unlikely that any bias large enough to show either unreal changes or hide real changes since previous surveys has arisen. Bias resulting from different net meshes being used will be noted where pertinent. Seines, 20 ft (6.1 m) and 50 ft (15.2 m) long of 0.5 inch (13 mm) stretched mesh, were used in the four reservoirs and in streams throughout the study period. Dipnets of fine mesh were used in the reservoirs. Synergized rotenone (Chem-Fish Special) was used in the river, creeks, and reservoirs.

The position of fishes in the gillnets (distance from shore and depth from cork line) were recorded. Fork length, weight, and sex were recorded from these fish shortly after capture. Scale samples were taken two to four rows above the lateral line, below the dorsal fin. Representative samples of gillnetted fish and all fish collected by other means were preserved for later examination.

Ages of fish are given from spawning time. Equations for length–weight relations are derived from eye-fitted regression lines plotted on double logarith-

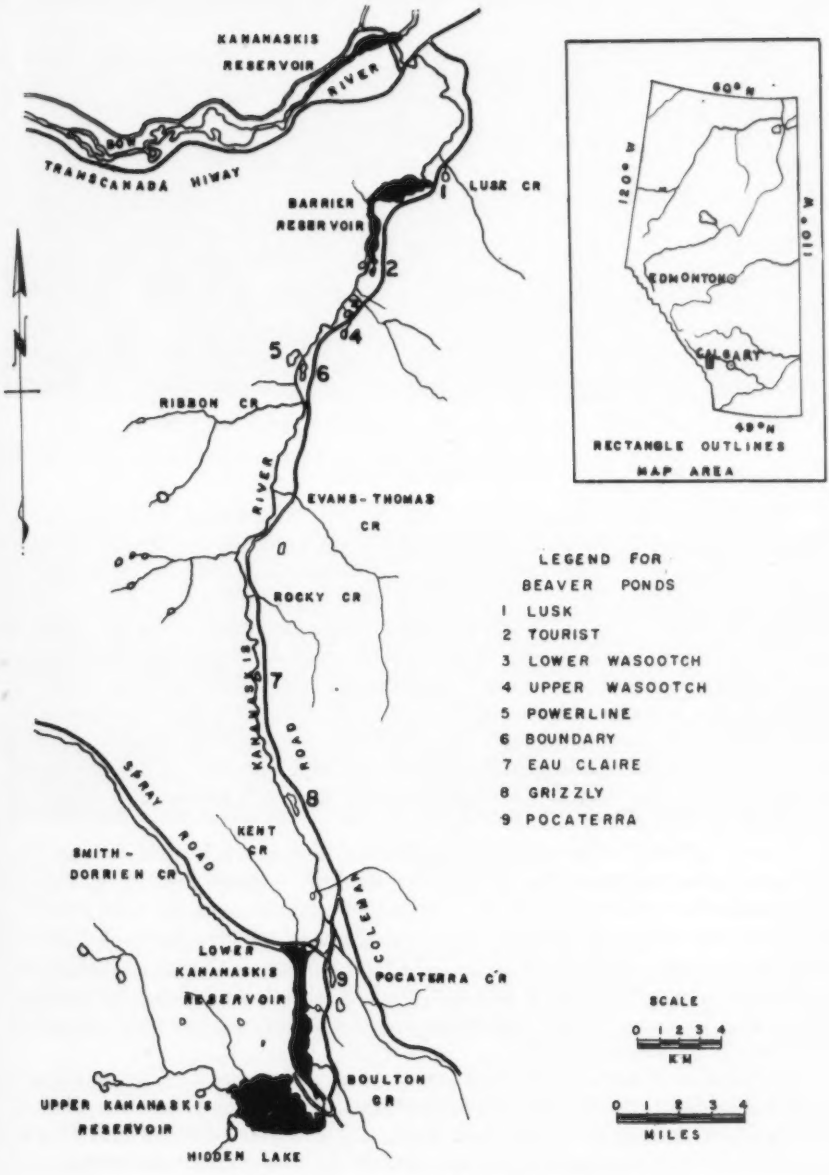

FIG. 1. Kananaskis River System and main roads.

mic graph paper and solved by the two-point method with simultaneous equations.

Former park wardens of the district provided information concerning the distribution of the fishes before 1936. Reports of fishery officers concerning the area were examined.

It was not possible to study an undisturbed system as a control. However, information on adjacent areas studied by Rawson (MS, 1948), some of which were undisturbed, has been utilized in this paper.

RESULTS

A comparison of past and present conditions in most of the Kananaskis system can be made in the geography, physicochemical conditions, plankton and benthos, and fish introductions from data collected since 1936. From the known changes that have occurred it will be possible to evaluate each factor as a cause of fish changes.

Geography and Power Development

The Kananaskis River lies in the Rocky Mountains in southwestern Alberta, 40 miles (64 km) west of Calgary. The river flows northward into the Bow River and is 52 miles (84 km) long from Upper Kananaskis Reservoir to its mouth.

A drop of 1325 ft (404 m) from Upper Kananaskis Reservoir to the river mouth has afforded development of several storage and hydroelectric projects by Calgary Power Ltd. Normally, Upper Kananaskis Reservoir (Fig. 2) is the first reservoir to be lowered in the winter to meet downstream water commitments. When that reservoir nears its low water level, Lower Kananaskis and Barrier (Fig. 3–5) Reservoirs are lowered respectively. Just prior to spring run-off, all three reservoirs are at their lowest level of the year. They are raised during the period of high spring run-off (Fig. 6).

Table I gives the physical characteristics of the reservoirs and data on the associated power plants. As opposed to the other three reservoirs, Upper Kananaskis Reservoir was utilized only for storage purposes for many years. Its outlet was deepened in 1932 to allow the original lake level of 5520 ft (1682 m) elevation to be lowered to 5513 ft (1680 m). Although earthfill had been placed over the outlet, it was not until 1936 that the full supply level of that time (5537 ft — 1688 m) was utilized. Only Upper and Lower Kananaskis Reservoirs occupy lake basins.

Kananaskis River is clear and fast flowing as are most of its tributary streams. The daily starting and stopping of Barrier and Pocaterra power plants, with irregular periods of longer shut down, cause marked fluctuations in the Kananaskis River water level. The lowering of the reservoirs in the winter and spring causes abnormally high water for this period of the year. The greatest volume was in late May and June in 1961, with 1700 ft^3/sec (48 m^3/sec) not uncommon.

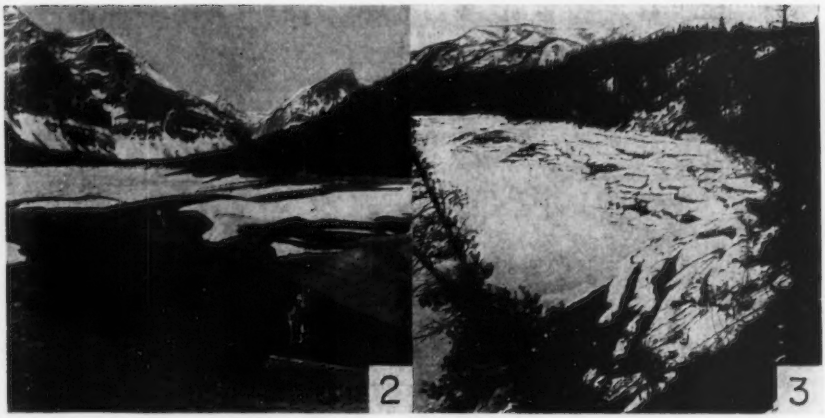

FIG. 2. Outlet end of Upper Kananaskis Reservoir at low water level, May 23, 1961.

FIG. 3. Shoreline of Barrier Reservoir at low water level. Note broken ice left behind at drawdown, May 9, 1961.

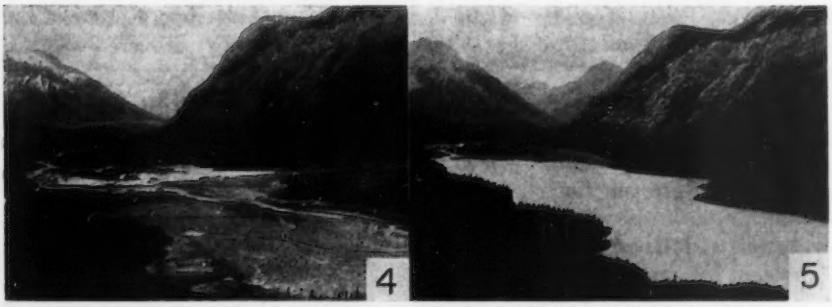

FIG. 4. Inlet end of Barrier Reservoir at low water level, May 1962.

FIG. 5. Inlet end of Barrier Reservoir at full supply level. August 1962.

Beaver ponds are numerous in the Kananaskis Valley.

Lower and Upper Kananaskis Reservoirs have many tributaries. Most streams entering the latter reservoir have falls at or near their mouth. Prior to impoundment short stretches of river existed between the falls and lake.

PHYSICOCHEMICAL CONDITIONS

TEMPERATURE

Kananaskis Reservoir is under the cooling influence of the Bow River. It is unlikely that an appreciable temperature change has occurred in this reservoir. Rawson (MS, 1948) gave the summer surface temperature range as 8.0–11.5° C.

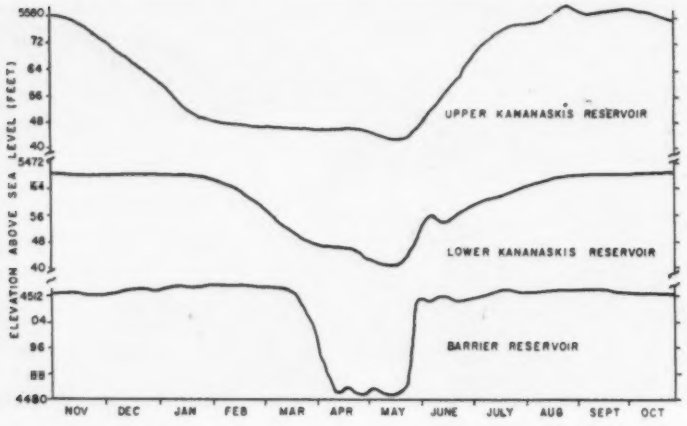

FIG. 6. Fluctuation in water level in Barrier, Lower and Upper Kananaskis Reservoirs from November 1960 to October 1961.

Barrier Reservoir provides a body of relatively warm water along the cold Kananaskis River (Fig. 7a, b). Midday surface shore temperatures in the summer ranged from 16 to 21° C. The reservoir generally is covered with ice from November to May.

Temperature series from Lower and Upper Kananaskis Reservoirs are included in Fig. 7b. Midday surface shore temperatures in Lower Kananaskis Reservoir ranged from 18 to 23° C while comparable temperatures in Upper Kananaskis Reservoir ranged from 17 to 22° C. Localized ice sheets were still present on both reservoirs at the end of May 1961. Temperature series taken in Lower and Upper Kananaskis Reservoirs since 1936 do not suggest any significant change.

Kananaskis River probably has had a lowering of temperature since 1955 with the outlet of Lower Kananaskis Reservoir beneath the thermocline during the summer months due to reservoir construction. Outlet water temperature seldom rose above 11.5° C. Surface drainage from Lower Kananaskis Lake prior to dam construction approached the lake surface temperature of about 15 or 16° C, but was subsequently cooled by tributaries to the Kananaskis River. The main channel of the river between Pocaterra plant and Barrier Reservoir varied from 8 to 10° C in May and 10 to 12° C in the summer of 1961. Side channels reached 12–14° C. Tributary streams had maximum temperatures of about 10° C. Beaver pond temperatures rose to a high of 24° C in the shallow zones and 22° C in the deeper areas.

OXYGEN CONCENTRATION

The large inflow and strong wind action keeps all the reservoirs well oxygenated. During 1961 no O_2 less than 5 cc/litre was observed. Nursall (MS, 1949) reported a low for the bottom O_2 of 4.0 cc/litre in January. Rawson

TABLE I. Physical characteristics of the reservoirs.

Reservoir and power plant	Location	Year water level first altered	Year power plant installed	Original area	Area full supply level	Area low water level	Volume reduction during drawdown
				acres	*acres*	*acres*	%
Kananaskis Kananaskis	51°05′N 115°04′W	1913	1913	–	202	201	unknown
Barrier Barrier	51°01′N 115°04′W	1947	1947	–	761	480	55
Lower Kananaskis Pocaterra	50°37′N 115°08′W	1955	1955	720	1600	700	60
Upper Kananaskis Interlakes	50°31′N 115°09′W	1932	1955	1440	2113	1565	unknown

Reservoir and power plant	Mean depth full supply level	Mean depth low water level	Maximum depth	Maximum drawdown	Forebay level at full supply level above mean sea level	Storage
	ft	*ft*	*ft*	*ft*	*ft*	*acre ft*
Kananaskis Kananaskis	6.5	unknown	unknown	4	4200	–
Barrier Barrier	47	40	128	34	4515	20,000
Lower Kananaskis Pocaterra	47	44.5	138	43	5469	50,000
Upper Kananaskis Interlakes.	unknown	unknown	328	52	5583	95,400

(MS, 1937) recorded a low of 3.8 cc/litre (53% saturation) for Lower Kananaskis Lake while Fillion (MS, 1963) found a low of 56% saturation on the bottom of the same body of water. It is unlikely that any significant change in the oxygen regime of the reservoirs occurred since 1936.

HYDROGEN ION CONCENTRATION

The pH of the Kananaskis system is generally alkaline (Table II). The consistent and relatively acid readings obtained by Miller (MS, 1954) and Thomas (MS, 1957) are in error (R. C. Thomas, personal communication) and are not included. No change in pH was noted during the flooding of Barrier Reservoir by Nursall (MS, 1949). The data (Table II) suggest no change in the pH of the Kananaskis system since 1936.

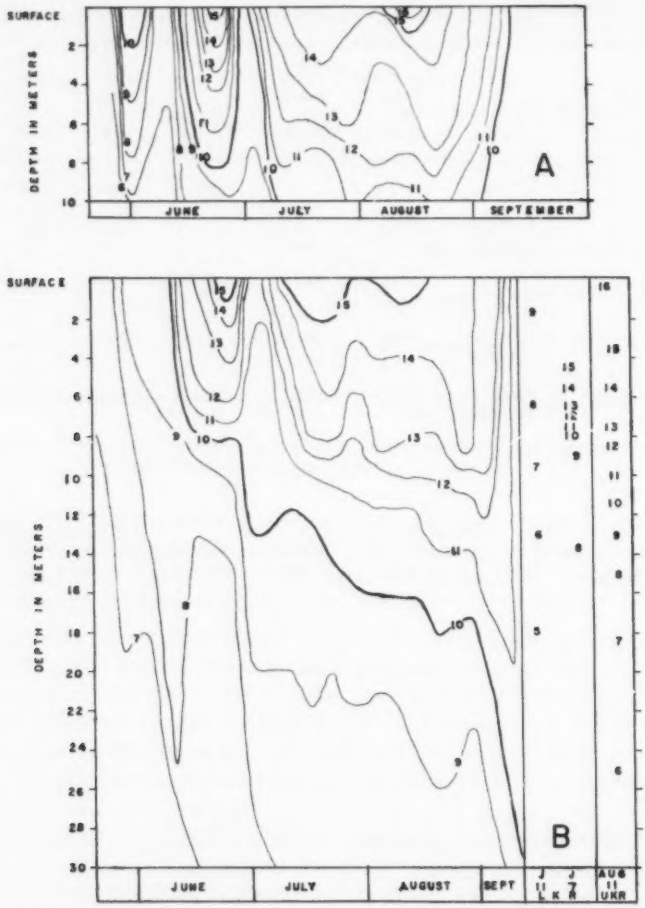

FIG. 7A. Summer 1961 distribution of isotherms in southern arm, Barrier Reservoir (temperature in degrees centigrade). B. Summer 1961 distribution of isotherms in eastern arm, Barrier Reservoir. June 11 and July 7 temperature series for Lower Kananaskis Reservoir (LKR) and August 11 series for Upper Kananaskis Reservoir (UKR) are included.

LIGHT PENETRATION

An index of light penetration obtained with Secchi disk showed the order of increasing transparency of the reservoirs was Kananaskis, Barrier, Upper Kananaskis, and Lower Kananaskis (Table III). Lower Kananaskis Reservoir lacked the silty tributaries that are common to Upper Kananaskis and Kananaskis reservoirs. Greater depth of the former allows silt to settle out that remains suspended in the latter. The large inflow into Barrier Reservoir also tends to keep silt in suspension. No shift in range of light penetration was evident from past studies of any reservoir.

TABLE II. Hydrogen ion concentration in the four reservoirs (1936–1961).

Reservoir	Site	1936	June 1947	July 1947	Aug. 1947	1948	June 1961	July 1961
Kananaskis	Surface		7.9		8.0			
	Bottom		7.9		8.0			
Barrier	Surface		7.9*	8.2*	8.1*	8.3*	8.2*	8.2*
	Bottom		7.8*	7.7*	7.8*	8.2*	8.1*	7.9*
Lower Kananaskis	Surface	8.1		8.1	8.2		8.0*	8.2*
	Bottom	7.7		7.9	7.9		7.7*	8.2*
Upper Kananaskis	Surface	8.0		7.8	8.1			7.9
	Bottom	7.7		7.5	7.6			7.6

*Denotes values averaged from two or more readings.

TABLE III. Secchi disk readings in meters for the four reservoirs (1936–1961).

	1936	June 1947	July 1947	July 1961
Kananaskis Reservoir	–	1.1	1.4	–
Barrier Reservoir	–	2.3*	3.0*	2.0*
Lower Kananaskis Reservoir	4.5	9.0	13.2	6.3
Upper Kananaskis Reservoir	2.2	6.0	8.5	3.3

*Denotes values averaged from two or more readings.

PLANKTON AND BENTHOS

Rawson (MS, 1948) described the Kananaskis Reservoir plankton as sparse and fluviatile. No data have been taken there since.

Nursall (MS, 1949) stated that establishment of plankton was rapid in Barrier Reservoir after its formation. The population was, and has remained, sparse (Nursall, MS, 1960). Fillion (MS, 1963) noted a substantial increase in the still relatively poor standing crop of bottom fauna since 1949. With the formation of Barrier Reservoir, a river fauna characterized by stoneflies

TABLE IV. Fish stocking in the Kananaskis system (1935–1961).

Year	Species			
	Rainbow trout	Cutthroat trout	Brook trout	Golden trout
1935	U. Kan. Res.	–	–	–
1936	–	Lower Kananaskis Lake Pocaterra Creek Boulton Creek	–	–
1937	–	–	–	–
1938	–	–	–	–
1939	–	Kananaskis River	–	–
1940	Foran Creek Ribbon Creek Kananaskis River Boundary Pond	–	–	–
1941	Upper Kananaskis Reservoir Ribbon Creek	–	–	–
1942	–	–	–	–
1943	Kananaskis River	–	–	–
1944	Upper Kananaskis Reservoir Lower Kananaskis Lake Pocaterra Creek Boulton Creek Kananaskis River	–	–	–
1945	Hidden Lake	–	–	–
1946	–	–	–	–
1947	Boulton Creek	–	–	–
1948	–	–	–	–

(*Continued*)

TABLE IV. Fish stocking in the Kananaskis system (1935–1961). (*Concluded*)

Year	Species			
	Rainbow trout	Cutthroat trout	Brook trout	Golden trout
1949	–	–	–	–
1950	Upper Kananaskis Reservoir	–	–	–
1951	–	Barrier Reservoir	Pocaterra Creek	–
1952	–	Moose Creek Muskeg Creek Evans-Thomas Creek Lusk Pond	–	–
1953	–	Rocky Creek	–	–
1954	–	–	–	–
1955	Pocaterra Lake	–	–	–
1956	Unknown pond	–	Boundary Pond	–
1957	–	–	–	–
1958	–	–	–	–
1959	Lower Kananaskis Reservoir	–	Lusk Pond Powerline Pond Upper Wasootch Pond	–
1960	–	–	–	Three Isle Lake
1961	–	North Galatea Lake	Powerline Pond Upper Wasootch Pond	Three Isle Lake

(Plecoptera) and mayflies (Ephemeroptera) has changed to a relatively richer benthic and planktonic fauna.

Little volumetric change in the plankton occurred since the damming of Lower Kananaskis Lake in 1955 (Thomas, MS, 1957). A marked decrease in the bottom fauna over the newly flooded area occurred but the original fauna

of the lake bottom remained unchanged from Miller's (MS, 1954) study. Fillion (MS, 1963) states that chironomids increased in numbers and dominate the flooded areas. Sphaeriids and oligochaetes were poorly represented in the flooded areas, but common at greater depths. R. C. Thomas (personal communication) states that Lower Kananaskis Lake lacked rooted aquatics before impoundment, as at present.

Rawson (MS, 1937) regarded Upper Kananaskis Reservoir as quite barren and less productive than Lower Kananaskis Lake. Fillion (MS, 1963) states that Lower and Upper Kananaskis Reservoirs are now similar in bottom fauna, the former reservoir being richer in standing crop. He gives evidence that the productivity is much less in both than would be expected in an unregulated lake under similar conditions and is probably less than pre-impoundment conditions.

Fish Stocking

Fish stocking has been carried out on a regular basis in the Kananaskis system since 1935 (Table IV). Accurate counts are not known for most plantings. The only recorded introduction of brown trout in the Bow system was made in 1925 at Carrot Creek, a tributary to the Bow River 21 miles (34 km) upstream (west) from the mouth of the Kananaskis River. Adult cutthroat trout were unsuccessfully introduced into Upper Kananaskis Lake in 1914 (J. Naylor, personal communication, former park warden). Previously barren, Ribbon Lake was stocked in 1920 with cutthroat fry. Gilmour (MS, 1950) states that trout from Lower Kananaskis Lake used in the past for restocking the Kananaskis lakes and other Alberta lakes were hybrids (*Salmo clarkii* × *S. gairdneri*). Reciprocal crosses were intentionally made at times for these plantings (J. Kovach, personal communication, former forestry warden). Introductions of lake chub, longnose dace, longnose sucker, and white sucker (probably all of Bow River origin) were made by anglers (evidence later).

Plantings of brook trout (*Salvelinus fontinalis*), rainbow trout (*Salmo gairdneri*), Atlantic salmon (*S. salar*), cisco (*Coregonus artedii*), lake whitefish (*C. clupeaformis*), smallmouth bass (*Micropterus dolomieui*), and mosquitofish (*Gambusia affinis*) were made in the Bow River upstream from the mouth of the Kananaskis River (Rawson, MS, 1939b; J. C. Ward, personal communication). Only the Atlantic salmon and smallmouth bass introductions were unsuccessful.

Biology of the Fishes

A knowledge of the past and present distribution and general biology of the Kananaskis fishes provides a further basis for establishing causes for the change in their abundance and distribution. In Fig. 8–17, "past" distribution denotes areas occupied by the species *only* in the past. It is based on the 1947 study of Rawson (MS, 1948) and the 1948–49 study by Nursall (MS, 1949). "Present" distribution denotes areas invaded or stocked *since* 1947 (1949 for Barrier Reservoir). "Past and present" indicates areas where the species

distribution has remained the same since 1936. "Rare" denotes areas where only a few specimens were taken in 1961. The presence of hybrid trout (*Salmo clarkii* × *S. gairdneri*) is not considered.

Table V shows the percentage occurrence of fishes gillnetted in the reservoirs from 1936 to 1961. When only one species is present, it will represent 100%. Introductions of other species lowered this percentage but did not necessarily lower the numerical abundance. Conversely, an increase in the percentage occurrence of one species may have been due to a numerical decrease in another. Conclusions regarding changes in abundance for species previously present thus were made only in cases where no new species had been introduced or where the original worker felt changes had occurred based on catch per unit effort. The nature of the data from past surveys preclude adequate statistical analysis. Seined fish in 1961 (Table VI) were generally less than 9 cm in fork length and were too small to be caught in the gillnets. Longnose suckers, 10–13 cm, outnumbered similar sized white suckers. This accounts for the increase in percentage occurrence in the 1961 from the 1960 gillnet catches of longnose suckers (Table V). In 1960, small-meshed gillnets were not used. Small white suckers, generally less than 9 cm, outnumbered the longnose suckers (Table VI). The species ratio of suckers in various year-classes thus varied considerably. All seine hauls and gillnet catches showed this relationship.

Several areas were extensively sampled without any fish being found. Marmot Creek (between Ribbon Creek and Powerline Beaver Pond), the Kananaskis River above Twin Falls (between Lower and Upper Kananaskis Reservoirs), a creek entering above Twin Falls, side-channels of the Upper Kananaskis River, and the outlet of Lyautey Lake (a tributary of Upper Kananaskis River), were poisoned without any fish being taken. It seems likely that Upper Kananaskis Reservoir was originally devoid of fish. Observations of J. Naylor and E. Stenton (former park wardens for the area) in the 1920s and early 1930s support this view.

MOUNTAIN WHITEFISH (*Prosopium williamsoni*)

Mountain whitefish occurred in the Kananaskis River to Pocaterra Falls (Fig. 8) and have remained unchanged in their distribution since the 1930s. This native fish is abundant in Kananaskis and Barrier Reservoirs and throughout the Kananaskis River. They were not found in tributaries to the river. Mountain whitefish outnumbered brown trout 10 to 1 in collections made each month with rotenone from a side-channel of the Kananaskis River 0.75 mile (1 km) south of Barrier Reservoir. In some sloughs, the two species were in equal numbers, but with brown trout occurring farther upstream. Along steep banks in Barrier Reservoir, young whitefish tended to stay close to the shoreline and bottom. They were rarely out from shore farther than 20 ft (6 m) and were commonly found near the bottom up to depths of 47 ft (14 m).

Schooling in mountain whitefish became evident at the age of 7 months (from November) in the river and reservoirs. Fry showed no tendency to hide but reacted to disturbances by swimming away. Schools tended to occupy small

TABLE V. Occurrence of different species gillnetted from 1936 to 1961 (percentage). P indicates presence noted by seining or nonquantitative gillnetting. Total number is for those species indicated quantitatively.

Reservoir	Year	Species											Total No.
		Mountain whitefish	Longnose sucker	White sucker	Brown trout	Rainbow trout	Cutthroat trout	Dolly Varden	Brook trout	Lake chub	Longnose dace	Brook stickleback	
Kananaskis	1938	76	2	–	–	–	7	15	–	–	–	–	55
	1947	54	–	3	22	11	–	7	3	–	–	–	28
	1961	40	3.5	40	16	–	–	0.5	–	P	P	P	157
Barrier	1947	47	–	–	–	9	24	20	–	–	–	–	156
	1948–49	P	–	–	–	P	P	P	–	–	–	–	–
	1960	68	6	18	8	–	–	–	–	–	–	–	289
	1961	48	24	17	8	2	0.5	–	–	0.5	P	–	1,093
Lower Kananaskis	1936	–	97.5	–	–	–	1	1.5	–	–	P	–	207
	1947	–	3	–	–	51	1	45	–	–	–	P	101
	1954	–	0.5	74	–	1	13.5 hybrids	11	–	–	–	P	223
	1961	–	4	83	–	4	–	6.5	–	2	0.5	P	160
Upper Kananaskis	1936	–	–	–	–	100	–	–	–	–	P	–	16
	1947	–	–	–	–	100	–	–	–	–	P	–	27
	1961	–	66	1.5	–	16	–	–	–	14	2.5	–	242

TABLE VI. Occurrence of different species seined in 1961 (percentage).

Reservoir	Species							Total No.
	Mountain whitefish	Longnose sucker	White sucker	Brown trout	Lake chub	Longnose dace	Brook stickleback	
Kananaskis	61	4	27	5	1	1	1	102
Barrier	40	22	36	0.9	1	0.1	–	1400
Lower Kananaskis	–	2	47	–	7	40	4	792
Upper Kananaskis	–	45	21	–	27	7	–	417

bays of the river with mud bottoms. They were often observed to be displaced by fluctuations in the river level, but this was probably of no harmful consequence. Over 95% of the whitefish had moved down the Kananaskis River and into Barrier Reservoir by the age of 20 months (July), thereby leaving only one year-class (0^+) abundant in the river above the reservoir. McHugh (MS, 1936) reported a downstream movement at a similar age in the unmodified Okanagan Valley of British Columbia.

Nursall (MS, 1949) reported a shift in the whitefish diet from allochthonous river organisms to autochthonous bottom forms (mainly chironomids) in Barrier Reservoir between 1947 and 1948 due to changes in abundance of these invertebrates. In 1961, chironomids were dominant in the diet of whitefish in Kananaskis and Barrier Reservoirs. River whitefish fed primarily on stonefly and mayfly larvae.

Most growth in immature whitefish occurred between June and September (Fig. 18). The 1961 growth rate of whitefish in both Kananaskis and Barrier Reservoirs has increased since 1947, and the former population had a faster growth rate than the latter in both years (Fig. 19). The first circulus on the scales was formed when fish were about 2.6 cm long (average of 7 months old). Kananaskis Reservoir whitefish are heavier per unit length than those in Barrier (Table VII). There was little overlap in the plotted points which suggests that the difference is significant.

In Kananaskis Reservoir all but one female were mature when 3 years 9 months. None was mature at 2 years 9 months. Approximately 50% of the males 2 years 9 months and all the males 3 years 9 months were mature. In Barrier Reservoir most females 3 years 11 months were ripe, while all males of the same age and about 30% of the 2 years 11 months group were ripe. Barrier Reservoir fish thus became mature at a smaller size than those in Kananaskis Reservoir but were approximately the same age. From distribution of fry, it is evident that considerable river spawning occurs. The presence of fully ripe and spawned-out females along the southern shore of the east arm of Barrier

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	1961	40	3.5	40	16	–	–	0.5	–	P	P	P	157
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	1948–49	P	–	–	–	P	P	P	–	–	–	–	–
	1960	68	6	18	8	–	–	–	–	–	–	–	289
	1961	48	24	17	8	2	0.5	–	–	0.5	P	–	1,093
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	1947	–	3	–	–	51	1	45	–	–	–	P	101
	1954	–	0.5	74	–	1	13.5	11	–	–	–	P	223
	1961	–	4	83	–	4	hybrids –	6.5	–	2	0.5	P	160
Upper Kananaskis	1936	–	–	–	–	100	–	–	–	–	P	–	16
	1947	–	–	–	–	100	–	–	–	–	P	–	27
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TABLE VII. Length–weight relationships of some fish in Kananaskis system.

Species	Location	Equation[a] (Log W =)
Mountain whitefish	Kananaskis Reservoir	3.039 logL–1.939
	Barrier Reservoir	3.029 logL–2.008
Brown trout	Barrier Reservoir	2.734 logL–1.620
Cutthroat trout	Lower Wasootch	2.545 logL–1.445
Rainbow trout	Kananaskis River	2.817 logL–1.597
	Upper Kananaskis Reservoir	3.171 logL–2.091
Longnose sucker	Barrier Reservoir	3.184 logL–2.105
	Upper Kananaskis Reservoir	2.968 logL–1.907
White sucker	Kananaskis Reservoir	2.959 logL–1.769
	Barrier Reservoir	3.107 logL–2.010
	Lower Kananaskis Reservoir	3.224 logL–2.164

[a]From equation LogW = $n$logL–b, where
W = weight in g
L = fork length in cm.

Reservoir suggests that beach spawning also occurs. Hatching probably occurs shortly before drawdown in the spring.

In Barrier Reservoir, mountain whitefish comprised about 85% of the sport-fish catch in 1961.

DOLLY VARDEN (*Salvelinus malma*)

Dolly Varden have become greatly restricted in their native Kananaskis range since 1947 (Fig. 9). They were once common in Kananaskis Reservoir but are now rare. A. Guinn (personal communication, fishing guide) reported them to be common in Barrier Reservoir and Kananaskis River and abundant below Pocaterra Falls until 1949. They are now rare in this area and remain as a self-sustaining population only in Lower Kananaskis Reservoir and Smith-Dorrien Creek.

Immature Dolly Varden (four age classes) in Smith-Dorrien Creek hid under rocks when disturbed. They were not observed to school.

Nursall (MS, 1949) noted there was an increase in the consumption of chironomids and river organisms and a corresponding decrease in the consumption of terrestrial organisms in Barrier Reservoir, from 1947 to 1949. Rawson (MS, 1937) reported Dolly Varden in Lower Kananaskis Lake to feed on suckers, stoneflies, and mayflies, while Miller (MS, 1954) stated that they fed primarily on leeches and amphipods, and to a lesser extent on young trout and suckers. In 1961, chironomids, the dominant organism of the reservoir, were the most abundant item in the stomach contents.

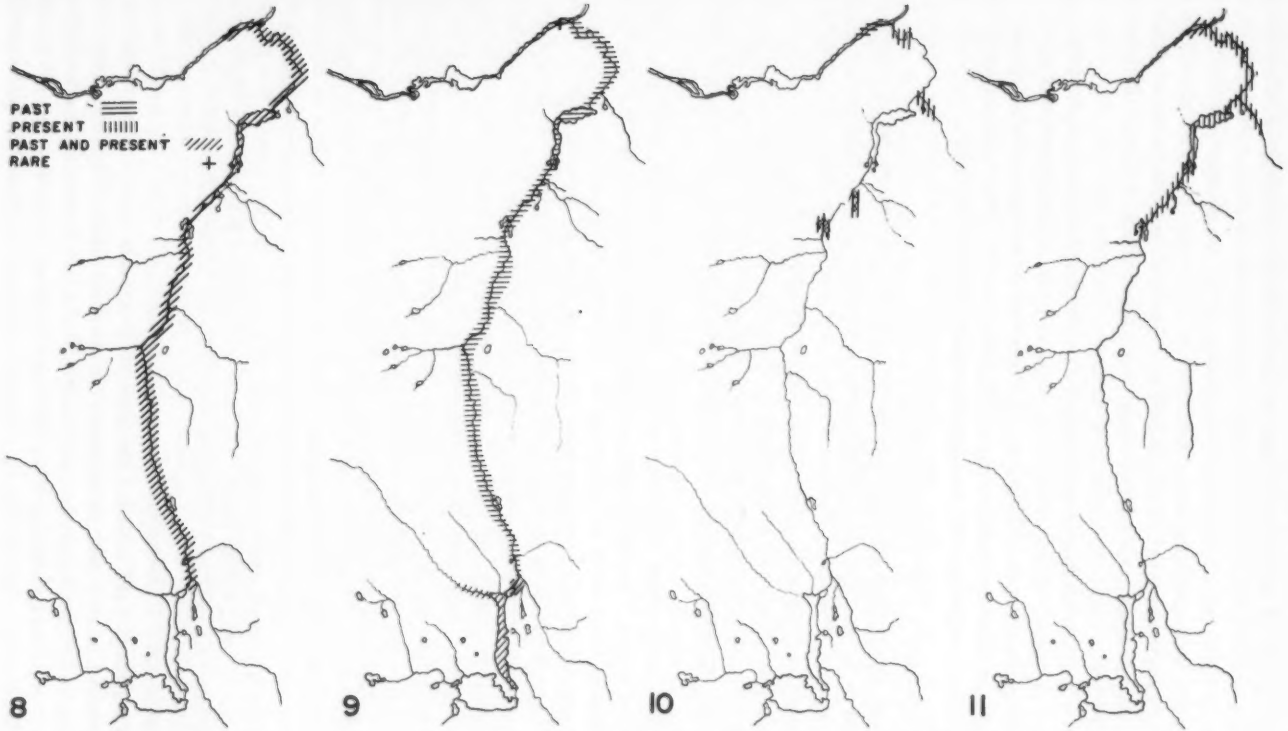

FIG. 8–11. Species distribution: Fig. 8, mountain whitefish (*Prosopium williamsoni*); Fig. 9, Dolly Varden (*Salvelinus malma*); Fig. 10, brook trout (*Salvelinus fontinalis*); Fig. 11, brown trout (*Salmo trutta*).

Fork length of immature groups of Dolly Varden in Smith-Dorrien Creek ranged from 3.1 to 4.2 cm at 9 months, 6.9 to 9.5 cm at 21 months, and 10.0 to 12.5 cm at 33 months. Number of specimens in these groups was 7, 6, and 4, respectively. One specimen measured 16.0 cm at 45 months. Difficulty in aging chars prohibited analysis of adult fish. R. C. Thomas (personal communication) found Dolly Varden up to 72 cm long weighing 4530 g in Lower Kananaskis Lake in 1954.

Dolly Varden do not reach maturity in the Kananaskis system until they are relatively large. Miller (MS, 1954) reported the majority mature at 5^+, or about 50 cm in length. All below 38.7 cm were immature. Nine specimens ranging from 33.7 to 46.0 cm were all immature in 1961. Only one female (49.5 cm) was maturing. No young were taken in the reservoir.

BROOK TROUT (*Salvelinus fontinalis*)

Brook trout were successfully stocked in several beaver ponds along the Kananaskis River (Fig. 10). Adults are common in Lusk, Upper and Lower Wasootch, and Powerline Beaver Ponds. Fry and fingerlings are present in Lusk Creek and Lusk Beaver Pond. Fry were observed in the beaver ponds stocked in 1961 (Table IV) and taken in Boundary Beaver Pond. They are rare in the Kananaskis River between Barrier and Kananaskis Reservoirs. Rawson (MS, 1948) reported them in Kananaskis Reservoir where they are now absent or rare.

Fry of brook trout hid under objects but not as readily as Dolly Varden and brown trout. They thus may compete with these two species for hiding places. Brook trout were not observed in compact schools.

Growth of brook trout appears to be good in all beaver ponds, except Lower Wasootch. Three mature males and three females taken in Lusk Beaver Pond in September varied from 21.3 to 25.0 cm in length. All were 35 months. Immature individuals, 0^+ and 1^+, were present. These are descendants of stream invaders. In Lower Wasootch Beaver Pond two mature males and two females varied from 14.0 to 17.2 cm in August. In Powerline Beaver Pond two mature males and one female varied from 24.5 to 27.0 cm in August. All were 2 years 10 months and survivors of the 1959 stocking. Brook trout fry were observed in Upper Wasootch and Powerline Beaver Ponds. They are survivors of the 1961 planting. Fry averaging 6.5 cm in length were taken in Boundary Beaver Pond in August and are offspring from a previously established population. Limited reproduction evidently occurs in some beaver ponds. Brook trout also spawn in Lusk Creek about 0.75 mile up from the Kananaskis River where 9-month-old (2.3-cm) and 21-month-old (6.5-cm) individuals were collected.

BROWN TROUT (*Salmo trutta*)

Brown trout have invaded the lower Kananaskis system after their introduction into Carrot Creek, a tributary to the Bow River (Fig. 11). They were first taken in Barrier Reservoir around 1949 (A. Guinn, personal communication) but were probably present by 1947, although undetected by Rawson (MS, 1948) and Nursall (MS, 1949). R. C. Thomas (personal communication) caught

adults in 1946 immediately below the present dam site. Fry and fingerlings are abundant in virtually all side-channels and sloughs of the Kananaskis River for 4.5 miles (7 km) above Barrier Reservoir. Extensive collecting showed that young brown trout do not occur farther upstream than this area. Adults have invaded the lower reaches of Powerline Beaver Pond, occur throughout Tourist Beaver Pond, and may ascend the river past Ribbon Creek. Brown trout have greatly increased their numbers and range in the Bow River reservoirs since their introduction and have spread at least 150 miles (241 km) down the Bow River from Carrot Creek.

In Kananaskis Reservoir brown trout fed on aquatic stoneflies, mayflies, and chironomids in 1961. Nursall (MS, 1960) gave their diet in Barrier Reservoir as consisting mainly of terrestrial insects, stream fauna such as caddis larvae, stoneflies, and mayflies, some chironomids, and small fish. In July 1961 the trout in the Barrier spillway pool fed heavily on dance fly adults (Empididae). Fry and fingerlings inhabiting sloughs at the head of Barrier Reservoir fed mainly on *Gammarus* and oligochaetes. Fry in the river had aquatic insects in their stomachs.

Figure 20 shows the summer growth of immature brown trout in the Barrier Reservoir area. Individuals from the outlets of Tourist and Powerline Beaver Ponds often showed exceptionally rapid growth rates and are omitted from the graph. The growth rate of brown trout in Kananaskis Reservoir exceeds that of Barrier Reservoir (Fig. 21) while both exceed the growth rate Rawson (MS, 1948) obtained for Gap Lake and Ghost Reservoir. The length–weight relationship for brown trout in Kananaskis Reservoir did not differ from that in Barrier Reservoir (Table VII).

It is evident from the distribution and abundance of fry that considerable spawning occurs in the Kananaskis River for 4.5 miles (7 km) above Barrier Reservoir. Some spawning also occurs in Lusk Creek, the Kananaskis River below Barrier Reservoir, and in Tourist Creek and Beaver Pond. The bottom of the beaver pond consists mostly of mud with little gravel, and is probably unsuitable for other trout to spawn in. It was not established at what age the brown trout mature. All individuals up to 32.5 cm (age 4+) were immature.

In Barrier Reservoir, brown trout formed about 7½% of the sport-fish catch in 1961.

CUTTHROAT TROUT (*Salmo clarkii*)

The native cutthroat trout of the Kananaskis system (Fig. 12) is virtually extinct. They were first recorded in Lower Kananaskis Lake by Williams (1921). Cutthroats were common, as were Dolly Varden, in the Kananaskis River and Evans-Thomas Creek in the 1930s and 1940s (J. Naylor and E. Stenton, personal communication) and in Barrier Reservoir until 1949 (Nursall, MS, 1949). In 1961, cutthroats or cutthroat-rainbow hybrids were common in several beaver ponds and creeks but were rare in Barrier Reservoir and the Kananaskis River. Lower Wasootch Beaver Pond may contain original Kananaskis cutthroat (planted stocks elsewhere are from Bow drainage). Rawson (MS, 1948) found

that the cutthroat trout in Lower Kananaskis Lake appeared to have been largely displaced by rainbow trout. He also reported a reduced number in Kananaskis Reservoir and other areas of the Bow River since 1938. They are now absent in Kananaskis and Lower Kananaskis Reservoirs.

Nursall (MS, 1949) noted an increase in chironomid larvae and river organisms, and a large decrease in terrestrial organisms in cutthroat stomachs between 1947 and 1949 in Barrier Reservoir.

Spawning probably occurs in June in Barrier Reservoir. In Lower Wasootch Beaver Pond all individuals over 17.6 cm had spawned that summer. The presence of various age classes shows that reproduction occurs in this pond. All individuals netted in Powerline Beaver Pond, varying from 21.0 to 29.5 cm, and Boundary Beaver Pond, varying from 17.8 to 21.2 cm, were 29 months old and had spawned that year.

RAINBOW TROUT (*Salmo gairdneri*)

Rainbow trout, following their introduction into Upper Kananaskis Reservoir in 1935, rapidly spread and were abundant throughout the Kananaskis system in the 1940s (Fig. 13). Once common, they are now rare in the Kananaskis River and Barrier Reservoir, and absent from Kananaskis Reservoir. Adults were once present in Grizzly Beaver Pond (A. Guinn, personal communication) but now are apparently absent. They show no signs of becoming extinct in Lower Kananaskis Reservoir as predicted by Miller and Paetz (1959).

Fork length for various age classes of rainbow trout on Kananaskis River above Pocaterra Falls in 1961 ranged from 2.3 to 3.0 cm at 2 months, 7.0 to 10.7 cm at 14 months, and 12.8 to 18.0 cm at 26 months. Number of specimens in these groups was 8, 13, and 16, respectively. One specimen measured 21 cm at 38 months. These specimens in addition to the Upper Kananaskis Reservoir population, were used to estimate length–weight relationships for rainbow trout (Table VII).

Streams suitable for trout spawning exist in Lower Kananaskis but not in Upper Kananaskis Reservoir. The presence of 1+ individuals (9.4–12.5 cm long) in Upper Kananaskis Reservoir indicates that successful spawning, likely beach spawning, does occur.

In Barrier Reservoir, rainbow trout comprised about 7½% of the sportfish catch in 1961. The Kananaskis River, and Lower and Upper Kananaskis Reservoirs were fished with the latter reservoir producing the highest percentage catch.

LONGNOSE SUCKERS (*Catostomus catostomus*)

Longnose suckers (Fig. 14), native to the Bow River, were observed in the Kananaskis River at the present site of Barrier Reservoir and in Smith-Dorrien Creek in the 1930s (E. Stenton, personal communication). They were undoubtedly introduced into Upper Kananaskis Reservoir where suckers were first reported present in 1952 (files of Fish and Wildlife Branch, Edmonton). Rawson (MS, 1948) noted a decrease in their numbers in Lower Kananaskis Lake between 1936 and 1947 as well as in several areas along the Bow River between

FIG. 12–15. Species distribution: Fig. 12, cutthroat trout (*Salmo clarkii*); Fig. 13, rainbow trout (*Salmo gairdneri*); Fig. 14, longnose sucker (*Catostomus commersonii*) and lake chub (*Hybopsis plumbea*). The presence of lake chub in Kananaskis Reservoir was first noted in 1961. White suckers occur immediately below Barrier Reservoir.

1938 and 1947. They have remained in low numbers in Lower Kananaskis Reservoir (Miller, MS, 1954; Thomas, MS, 1957) (Table VI). Longnose suckers occur in the Kananaskis River below Barrier Reservoir but not above it.

Suckers in Upper Kananaskis Reservoir were somewhat lighter per unit length than those of the Barrier Reservoir population (Table VII). Fork length of immature longnose suckers in Upper Kananaskis Reservoir was found to range from 1.4 to 2.0 cm at 1 month, 3.5 to 4.6 cm at 13 months, 6.0 to 8.1 cm at 25 months, 8.6 to 9.5 cm at 37 months, and 12.0 to 14.0 cm at 49 months. Number of specimens in each group were 90, 100, 100, 100, and 30, respectively. Rawson (MS, 1937) noted that individuals in Lower Kananaskis Lake did not exceed 30 cm. In the present survey many adults between 30.0 and 35.5 cm were caught. No evidence of a stunted population was noted.

In Barrier Reservoir ripe males and females were first taken on June 6 with the first spawned-out individuals caught on June 26. Beach spawning is likely common in the Kananaskis system.

Ligula commonly made up 20% of the body weight in 2^+, 3^+, and 4^+ individuals in Upper Kananaskis Reservoir. It was less abundant as a parasite of suckers in Lower Kananaskis Reservoir and rare in the others.

WHITE SUCKERS (*Catostomus commersonii*)

White suckers (Fig. 15) were not reported in the Kananaskis system by Rawson (MS, 1948) or in Barrier Reservoir by Nursall (MS, 1949). They were first noted by Miller (MS, 1954) and Nursall (MS, 1960) in Lower Kananaskis and Barrier Reservoirs, respectively, and were numerous at that time. White suckers occur in the Kananaskis River below Barrier Reservoir and may have entered the latter prior to impoundment, but were undoubtedly introduced into Lower Kananaskis Lake and Upper Kananaskis Reservoir as live bait. They have increased in relative numbers in Kananaskis Reservoir from when they were first reported present in 1947. Rawson (MS, 1948) also noted an increase in their abundance in other Bow River areas between 1938 and 1947. They are native in the Bow River but do not occur as far upstream as longnose suckers.

In Barrier Reservoir, the first ripe male was noticed on May 13, while most males were ripe on June 6 and females on June 19. Spawned specimens were first taken June 26 with some spawning as late as July 20. In Lower Kananaskis Reservoir ripe males and females were taken on June 10. By July 7 all had completed spawning. The late spawning of both suckers in the reservoirs, especially in Barrier, probably is a result of the cold temperature. In landlocked lakes of Alberta white suckers must beach spawn and likely only do so in the Kananaskis system. In 1961, white suckers reached spawning size at 31 cm (5^+) in all the reservoirs.

Ligula infections were common only in Lower and Upper Kananaskis Reservoirs.

LAKE CHUB (*Hybopsis plumbea*)

Lake chub (Fig. 15) were first noted in the Kananaskis River system during the present study. They were found to be abundant in Lower and Upper

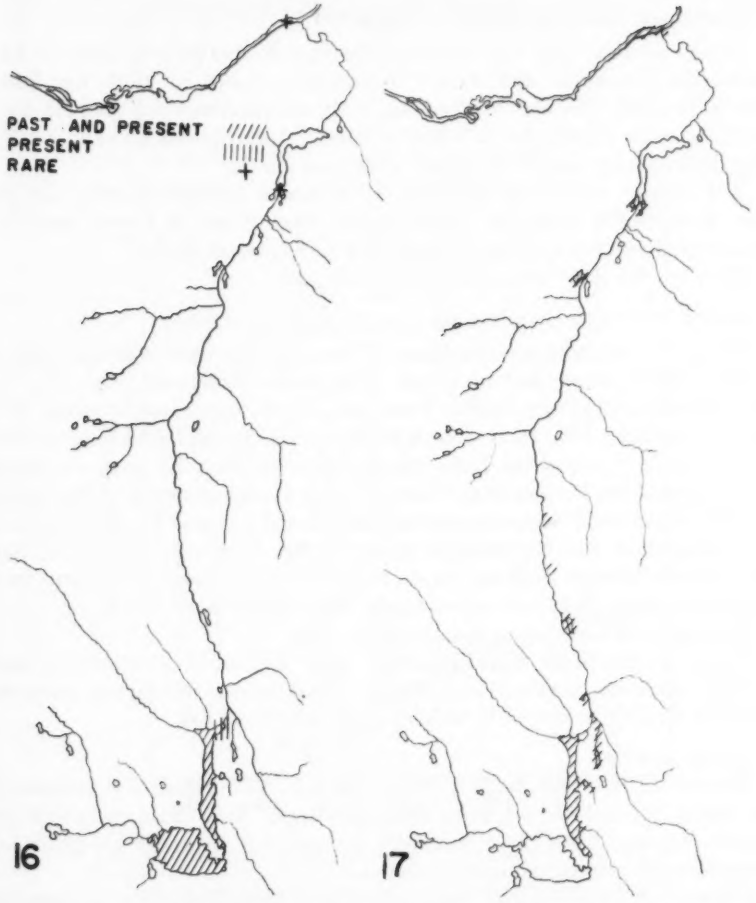

FIG. 16–17. Species distribution: Fig. 16, longnose dace (*Rhinichthys cataractae*); Fig. 17, brook sticklebacks (*Culaea* (= *Eucalia*) *inconstans*).

Kananaskis Reservoirs, rare in Kananaskis and Barrier Reservoirs, and absent from all streams and beaver ponds. Lake chub were undoubtedly introduced as bait from a Bow River stock. They are not native to the upper Bow River.

Lake chub commonly occurred in schools with suckers and longnose dace.

The largest individuals were slightly over 13 cm (5+). In Upper Kananaskis Reservoir in July many females (3+) were maturing. Ripe individuals were noted on August 8 and 9 in Lower and Upper Kananaskis Reservoirs, respectively.

Hybrids of *Hybopsis plumbea* × *Rhinichthys cataractae* were taken in Upper Kananaskis Reservoir.

LONGNOSE DACE (*Rhinichthys cataractae*)

Longnose dace (Fig. 16), native to the Bow River, were reported in Upper Kananaskis Reservoir and Lower Kananaskis Lake in 1936 by Rawson (MS, 1937) where they are still common. Only one specimen was taken in Barrier and Kananaskis Reservoirs in 1961. Dace were absent in the Kananaskis River except immediately below Pocaterra Falls and plant.

The largest individual taken in 1961 was a 115-mm female (5+) from Upper Kananaskis Reservoir. Ripe females were taken in Lower and Upper Kananaskis Reservoirs as late as August 8 and 9, respectively.

Many of the dace were infected with *Ligula*.

BROOK STICKLEBACKS (*Culaea* (= *Eucalia*) *inconstans*)

Brook sticklebacks are abundant in many of the beaver ponds adjoining the Kananaskis River and in Lower Kananaskis Reservoir (Fig. 17). They occur throughout Grizzly Beaver Pond and in swampy areas adjacent to the ponds but isolated from them except in the spring. Sticklebacks have probably entered Lower Kananaskis Lake through introduction by man or through swampy areas from Pocaterra to Boulton Creek at high water. It seems unlikely that this species could have surmounted the falls and gorge at Pocaterra although they occurred in the Kananaskis River at the mouth of Pocaterra Creek. They are not always confined to or found in accessible areas where heavy vegetation occurs. They are native to the Bow River above Banff.

Ripe females were taken from June to July.

Many populations were infected with *Ligula*. Exceptionally heavy infections occurred in Pocaterra Beaver Pond where the larvae commonly formed up to 35% of the body weight of an individual fish.

OTHER SPECIES

Rawson (MS, 1939a, b, MS, 1948) and J. C. Ward (personal communication) report the following species from the upper Bow drainage which have not been recorded from the Kananaskis system. All distances are given from the mouth of the Kananaskis River.

Cisco (*Coregonus artedii*) were successfully introduced into Minnewanka Reservoir (25 miles (40 km) upstream) and planted (1953) in Spray Reservoir (15 miles (24 km) upstream). Lake whitefish (*Coregonus clupeaformis*) were successfully introduced in Minnewanka Reservoir. Lake trout (*Salvelinus namaycush*) occur in Minnewanka Reservoir and in Ghost Reservoir (15 miles (24 km) downstream). Northern pike (*Esox lucius*) occur in Glenmore Reservoir (45 miles (72 km) downstream). Flathead minnow (*Pimephales promelas*) occur in Bighill Creek (30 miles (48 km) downstream). Blacknose dace (*Rhinichthys atronasus* (= *atratulus*)) were reported in Lake Minnewanka by Rawson but their presence requires further confirmation. Mosquitofish (*Gambusia affinis*) were successfully stocked near Banff in hot springs (36 miles (58 km) upstream). Trout-perch (*Percopsis omiscomaycus*) are found in Jumpingpound Creek (29 miles (47 km) downstream). Burbot (*Lota lota*) occur in the Bow River near Banff and in Ghost Reservoir.

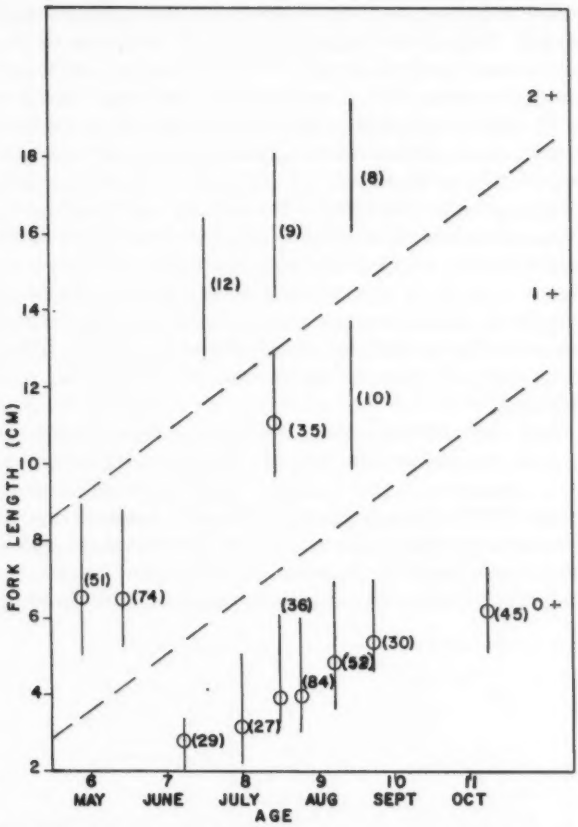

FIG. 18. Summer growth of immature mountain whitefish in Barrier Reservoir, 1961. Mean length given for large samples. Sample size in parentheses. Age in months from November.

DISCUSSION

Mountain whitefish showed no sign of being unfavorably affected by hydroelectric development or by other species of fish, and their growth rate has increased since 1947. Lack of a hiding reaction to disturbances does not bring them into competition with brown trout and an early maturity with relatively long life aid in their success.

Mountain whitefish remained successful in their native range, whereas Dolly Varden became rare due to the following: (1) displacement by brown trout where their ranges overlapped; (2) competition with white suckers in Lower Kananaskis Reservoir. Rawson (MS, 1948) reported an increase in Dolly Varden from 1936 to 1947 associated with a decrease in longnose suckers and a subsequent decrease in Dolly Varden associated with an increase in white

suckers. Studies demonstrating the effect of suckers on salmonids are unfortunately lacking. This study suggests that the presence of suckers had a detrimental effect on Dolly Varden; (3) relative old age and large size before maturity and high vulnerability to anglers. Catchable size is reached well before maturity. Sport fishing is popular in all the reservoirs and particularly large catches were made during reservoir construction and during World War II in the vicinity of Barrier Reservoir; (4) changes in spawning area. Since 1942 and 1955 the Kananaskis River below Twin Falls and Boulton Creek, respectively, were lost as significant spawning areas. However, Smith-Dorrien Creek was added and probably became the main spawning stream.

Brook trout were likely displaced from Kananaskis Reservoir by brown trout. The population density of brown trout was not sufficiently high to have displaced them from Lusk Creek and from Powerline Beaver Pond. There was, however, no suggestion that the brook trout and rainbow trout adversely affected one another.

Brown trout fry and fingerlings are well adapted to fluctuating rivers. They have a fixed territory-holding habit during rising water but seek open water during a decreasing water velocity, thereby avoiding capture in shore pools (Lindroth, 1955). Among young salmonids, brown trout dominated in competition for space. They generally select the most favorable position in rivers and are successful in aggressively defending their territory from intruders (Kalleberg, 1958). Adults may be more passive. It seems likely that

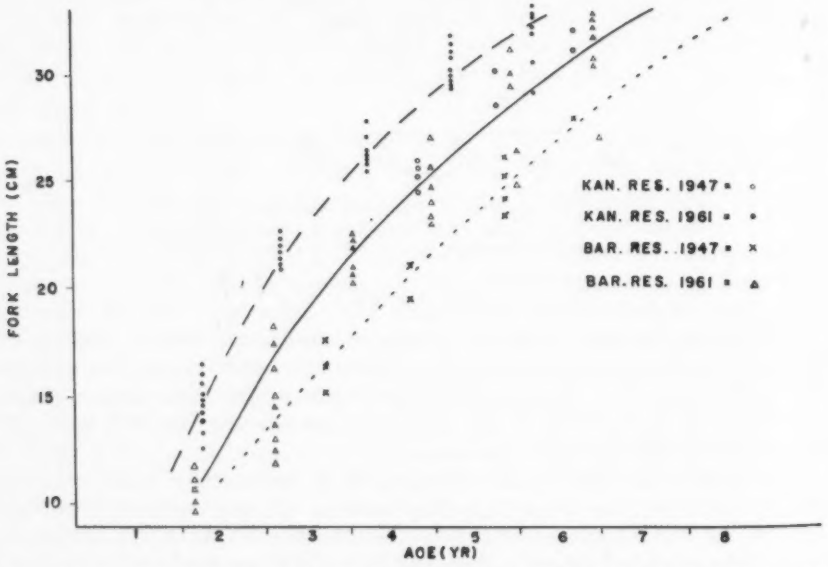

FIG. 19. Growth of mountain whitefish in Kananaskis and Barrier reservoirs. (Kananaskis Reservoir 1961 = — — —; Kananaskis Reservoir 1947, Barrier Reservoir 1961 = ———; Barrier Reservoir 1947 = ...). Rawson's 1947 data modified.

brown trout caused the marked decrease in Dolly Varden, brook trout, cutthroat trout, and rainbow trout where their ranges overlapped. Introduced brown trout were noted to displace native trout throughout North American inland waters (e. g. Fenderson, 1954, for Maine; R. J. Naverre, personal communication, for new Mexico; and Northcote, MS, 1960, for western North America). Wiggins (MS, 1950) and K. Radway Allen (personal communication) cite some instances to the contrary. Worthington (1941) notes that brown trout held their own in Great Britain against introduced rainbow trout. Brown trout are difficult to catch by most anglers (Andriano, 1960; Fenderson, 1954; Miller, 1957; Northcote, MS, 1960; Wiggins, MS, 1950; and Worthington, 1941) and this also aided in success of this fish.

Runnström (1964) noted the long-term effects in Swedish reservoirs of brown trout and char relative abundance to be the opposite to those of this study. Both numbers and growth of brown trout decreased but only growth decreased in char (*Salvelinus alpinus*), a closely related species to Dolly Varden. Differences in the physical environment at spawning time and the supply of food between Swedish reservoirs (Nilsson, 1964; Runnström, 1964) and those of this study may explain this difference.

The native Kananaskis cutthroat trout is extinct, except possibly in Lower Wasootch Beaver Pond. It is likely that cutthroat trout hybridized with introduced rainbow trout. Their observed decrease in Lower Kananaskis Lake from 1936 to 1947 corresponds with an increase in rainbows and concurrent

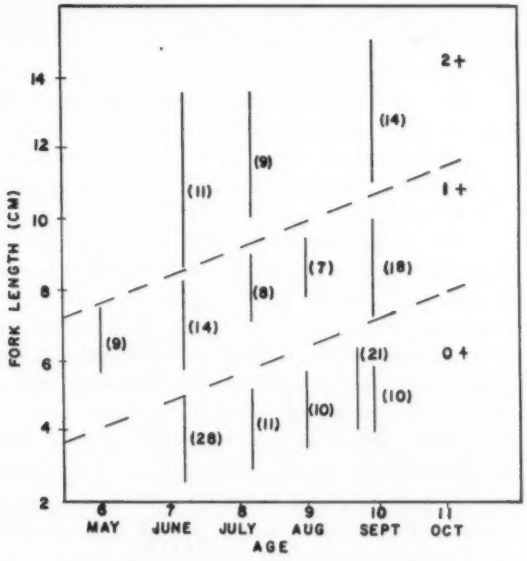

FIG. 20. Summer growth of immature brown trout in Barrier Reservoir and Kananaskis River 1961. Sample size in parentheses. Age in months from November.

hybridization. Brown trout and suckers probably also had a detrimental effect on cutthroats where their ranges overlapped. Cutthroat trout survived in beaver ponds where rainbow and brown trout are not present and in Powerline Beaver Pond.

Miller (1953) suggested that tributaries do not act as nurseries for main streams but have resident populations of their own. The reduction or elimination of Dolly Varden and cutthroat trout in tributaries of the Kananaskis River suggests that feeder streams may sometimes be dependent on the main streams.

Rainbow trout have probably decreased in numbers since 1947 because of: (1) displacement by brown trout where their ranges overlapped, as previously discussed; (2) cooling in the upper reaches of the Kananaskis River, since 1955, below the critical holding temperature to which the fry were adapted. Cartwright (1961) and Northcote (1962) present evidence that in some rivers rainbow trout fry cannot hold in currents when the water temperature is below 13 or 14° C; (3) competition with white and longnose suckers. Although Rawson and Elsey (1950) did not observe an improvement in the rainbow trout fishery of Pyramid Lake, Alberta, after heavy netting of suckers for 5 years, a large population of suckers was still present as well as predacious lake trout. Trout and suckers may also be in a closer relationship in Kananaskis Reservoirs; (4) decreased spawning area in Lower Kananaskis Reservoir. Beach spawning in Lower

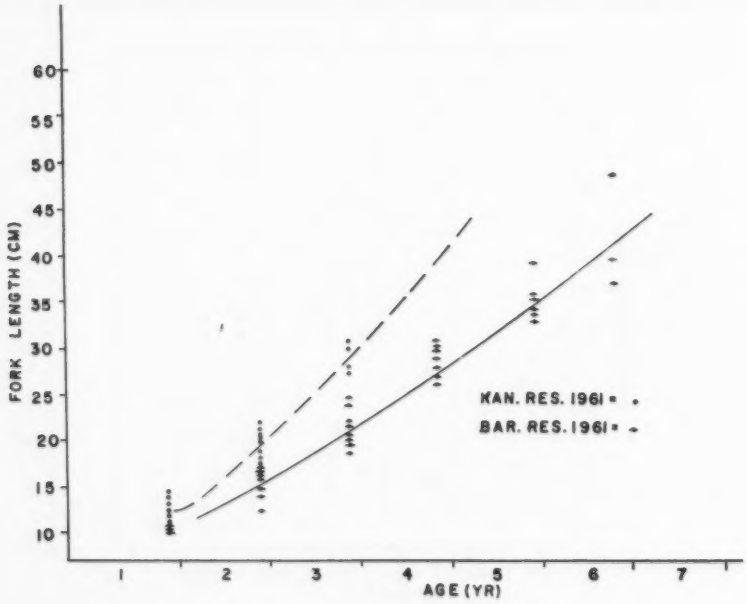

FIG. 21. Growth of brown trout in Kananaskis and Barrier Reservoirs. (Kananaskis Reservoir 1961 = — — —; Barrier Reservoir 1961 = ———).

Kananaskis Reservoir is likely insignificant but appears to be the main source of reproduction in Upper Kananaskis Reservoir.

The steady decrease in longnose suckers between 1936 and 1947 (Rawson, MS, 1948), and substantiated by gillnet catches by hatchery workers for trout between 1942 and 1947, in Lower Kananaskis Lake was attributed by Miller (MS, 1954) to the destruction of the major spawning inlet, the Kananaskis River below Twin Falls. This fails to explain the decrease in longnose suckers, approximately concurrent with an increase in white suckers, in Lac des Arcs and Gap Lake which had relatively stable physical conditions. Furthermore, longnose suckers are capable of beach spawning, as in Barrier and Upper Kananaskis Reservoirs. Also the decline of longnose suckers from 1936 to 1947 in Lower Kananaskis Reservoir was associated with an increase in rainbow trout and Dolly Varden. Both are inlet spawners (the former was stocked only once in this time period, the latter not at all). Increases in trout and char have not previously been noted to affect suckers and no factor can be suggested which would cause a decrease in longnose suckers with an increase of trout and char. There is evidence that temperature may be important in determining the ratio of sucker species. M. Atton (personal communication) states that longnose suckers prefer colder and deeper waters than white suckers. The geographic distribution of longnose and white suckers also suggests that the former is more tolerant of cold waters. These generalizations, however, cannot explain the decrease of longnose suckers in Lower Kananaskis Lake. Longnose and white suckers occur together in large numbers in many lakes in British Columbia (unpublished observations 1962–64). Stunted populations of longnose suckers occur in Hart and Bear Lakes (11–13 and 15–19 cm fork length, respectively, when spawning) of the Peace River drainage along with white suckers (22–33 cm fork length when spawning). Both spawn on beaches in these two lakes. Both are also abundant in relatively shallow and deep lakes without either species being stunted. Furthermore, since longnose suckers appear to have decreased before white suckers increased in Lower Kananaskis Lake, the succession could not have been due to competition. Competition may occur, however, since different ratios in numbers of various age classes in Barrier Reservoir exist, although different spawning successes dependent upon the physical environment could explain this. Rawson's (MS, 1937) finding that longnose suckers over 30 cm long were not present or were rare in Lower Kananaskis Lake indicates they matured at a smaller size then than now. An increase in their growth rate has likely occurred. A common cause may not exist for the observed sucker changes in relative abundance for the various reservoirs, and more information is needed to arrive at a suitable explanation for this intriguing phenomenon.

A large number of young suckers compared to adult suckers in Upper Kananaskis Reservoir, relative to other areas, is suggestive of an expanding population, probably due to the recency of their introduction. They have probably reached stable numbers in the other reservoirs. Longnose suckers were apparently never abundant in two Bow River reservoirs, Kananaskis and Horseshoe.

White suckers following their introduction into the various reservoirs greatly increased in numbers after 1947 to become one of the dominant species, except in Upper Kananaskis Reservoir, as they did in Lac des Arcs and Gap Lake between 1938 and 1947. They have been abundant in Ghost Reservoir but rare in Horseshoe Reservoir since at least 1938. As noted previously, white suckers increased in several areas, impounded and undisturbed alike, following a decrease of longnose suckers. In Kananaskis Reservoir they also increased where longnose suckers were apparently never abundant. Again, a suitable explanation for these changes cannot be given. Trautman (1957), however, noted that white suckers were exceptionally generalized in their habits and tolerant of many conditions.

Lake chub and longnose dace occupy a wide variety of habitats over their total range. The absence of these two cyprinids from the Kananaskis River and its tributaries suggests that both were introduced, probably as bait. There is little doubt from past surveys that lake chub were recently introduced into the system. The presence of longnose dace was noted at Banff and Calgary by Nichols (1916). However, the absence of relict longnose dace populations outside the reservoirs and the observations of early park wardens suggests that this fish was introduced to the Kananaskis system prior to 1936.

Brook sticklebacks were more numerous in beaver ponds than in other bodies of water. The seasonally changing habitat of Lower Kananaskis Reservoir has not caused their extinction. Their failure to colonize Barrier Reservoir, despite their occurrence in sloughs at the head end, may be due to the relatively rugged, steep shoreline and silty water which does not provide a suitable environment.

Elton (1958) observed that invaders were most likely to become dominant in simplified and unstable environments. Perhaps the increase in brown trout, white suckers, and lake chub over other species would not have occurred had stable environmental conditions existed. However, changes in the species of the Kananaskis system have also been observed in physically stable environments. The species new to the Kananaskis system have thus successfully established themselves in new niches, often to the detriment of previously established species.

In Sweden, construction of fluctuating reservoirs increased plankton-feeding fish and decreased bottom-feeding and piscivorous fish. This generalization is poor for the Kananaskis system, because of the great abundance of chironomids. Nilsson (1955) found that chars in Swedish lakes fed on plankton in open water when the bottom food was scarce. Such a change was not observed in any Kananaskis salmonid, due probably to abundance of chironomids. Larkin (1956) showed that cold-water fishes have generalized feeding habits and have a relatively wide tolerance of habitat type. Although preference is shown when there is a variety of food, they generally eat what is available. Thus, food changes were considered relatively unimportant for changes in each fish species. Importance of certain food items, however, may have been overlooked (Johannes

and Larkin, 1961) and certainly the decline of many invertebrates, as noted by Fillion (MS, 1963), keep down the fish biomass from what it otherwise might be.

Lagler (1956) stated that most fish tolerate both wide ranges and rapid changes of acidity and alkalinity and that trout live in waters of greater $[O_2]$ than 4 or 5 ppm (2.8–3.5 cc/litre). It is therefore unlikely that pH and $[O_2]$ had any selective influence on the fishes. Indeed there is evidence that the pH and $[O_2]$ were not altered. None of the fish approached high temperatures that are lethal as given by Black (1953), Brett (1944), and Wiggins (MS, 1950).

Dogiel *et al.* (1958) noted a lowering in the abundance of parasites immediately after flooding a reservoir, with a subsequent rise in numbers. Parasites unlikely caused any changes in the fish population, but changes in fish and invertebrate fauna would cause changes in the parasite fauna.

Construction of reservoirs for hydroelectric power changed the physical environment and contributed to the observed changes in the species composition. Decrease in game species was, however, largely due to the introduction of new species and sport fishing. These factors by themselves would probably have created a situation similar to now and to Lower Kananaskis Lake in 1936 when longnose suckers predominated. Other factors remained relatively constant.

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Browning of Freeze-Dried Fish[1]

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ABSTRACT

Lingcod (*Ophiodon elongatus*) and sole (*Parophrys vetulus*) muscle were treated in different experiments with a dilute solution of ribose, or with a particulate enzyme preparation capable of oxidizing both ribose and glucose before freeze-drying at low temperature. Freeze-drying did not occasion loss of free glucose or ribose. Various experiments in which the freeze-dried fish was stored at −30, 25, 33, or 37°C and relative humidities (RH) of 0, 5, and 10% showed that browning proceeded rapidly at all RH values except in samples held at −30°C, and developed more rapidly at the higher temperatures. Browning was accelerated in samples containing added ribose, and retarded in those in which a large proportion of the sugars was removed by enzyme treatment. Storage in nitrogen retarded development of browning but did not eliminate it, indicating that oxidation is responsible for much of the discolouration.

INTRODUCTION

The preparation and storage properties of freeze-dried meats have been discussed in reviews by Sharp (1953) and Harper and Tappel (1957). While it appears that acceptable products are readily obtained, the method undoubtedly suffers several serious drawbacks. The cost of freeze-drying is rather high, the blood pigments of meats tend to become brown due to formation of methaemoglobin, and some toughness may develop on storage. However, freeze-dried beef with 3% water content, when properly packaged in moisture-proof and oxygen-proof containers and stored 9 months at 70°F, was judged as acceptable as similar freeze-dried meat stored at 0°F (Harper and Tappel, 1957).

Jones (1954, 1959) observed that browning reactions occurred in freeze-dried extracts of cod muscle, even at 0% relative humidity (RH). However, these were not accompanied by loss of free sugar and were traced to degradation of 1-methylhistidine. Jones also noted that stored freeze-dried fish muscle became brown when stored at 0% RH.

Recent Japanese work has suggested that oxidation of the oil is largely responsible for the brown discolouration of freeze-dried dark muscle of horse mackerel (*Trachurus japonicus*). On the other hand, discolouration of freeze-dried white muscle of this species was stated to be due to Maillard reactions. Such factors as the role of free sugars, of storage in nitrogen, and of the possible influence of storage RH were not studied by these investigators (Toyomizu *et al.*, 1963).

Past work in these laboratories has emphasized the importance of ribose and glucose in occasioning Maillard browning in heated fish flesh (Tarr, 1954)

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and has shown that removal of these sugars markedly decreases browning (Tarr and Bissett, 1945; von Tigerstrom and Tarr, in press), but has not involved freeze-dried fish. The lack of information on Maillard or other browning reactions in stored freeze-dried fish prompted the present investigation.

MATERIALS AND METHODS

Lingcod (*Ophiodon elongatus*) and lemon sole (*Parophrys vetulus*) were obtained while in rigor and were promptly frozen and stored at −30°C for several weeks. Prior to use the fish were partially thawed at room temperature and cut into portions which were approximately 10 g in weight and 6 mm thick. These portions were stored 65 hr at 0°C to permit free ribose and glucose to form (Tarr and Leroux, 1962). In some experiments a one in ten aqueous dilution of a particulate enzyme preparation from *Pesudomonas fragi* (Leroux and Tarr, 1963) was added to certain samples, and these were held 48–72 hr at 0°C in order to permit removal of ribose and glucose by oxidation. In previous work this enzyme preparation was used successfully to remove sugars from fish muscle and thus reduce Maillard browning after heating (von Tigerstrom and Tarr, in press). In some instances a solution of ribose was added to increase the natural sugar content. After the pretreatments, the fish portions were frozen in air at −30°C and freeze-dried on trays at approximately 50 μ in a Virtis freeze drier without application of heat. The dry samples were promptly transferred to plastic dishes in desiccators which contained magnesium perchlorate or sulphuric acid solutions capable of maintaining RH values of 0, 5, or 10%. Control samples were stored in polyethylene bags at −30°C.

Glucose and ribose were determined as follows. Representative pooled samples of fresh, or freeze-dried fish after reconstituting with water, were blended at 0–3°C with 2 volumes of 0.6M perchloric acid. The perchloric acid was removed as usual (Tarr and Leroux, 1962) and an aliquant of the clear extract representative of 10 g of fish was run slowly through two superimposed 10 × 1.9 (diam) cm columns containing Dowex 1 × 10 Cl^- (200–400-mesh) and Dowex 50 × 4 H^+ (100–200-mesh) resins. The columns were washed with 100 ml of water and the eluates evaporated to 5–10 ml using rotary evaporators with an external bath temperature of 30°C. The glucose and ribose content of the solutions was determined by the anthrone and orcinol reactions as in previous work (Tarr and Leroux, 1962). The solutions were free from nucleosides and nucleotides which, if present, would interfere with ribose determinations.

The particulate enzyme of *P. fragi* was prepared as before (Leroux and Tarr, 1963), and it was promptly frozen and stored at −30°C. It contained 14.7 mg of protein N/ml and, when used in 1:10 aqueous solutions, oxidized 11.9 μg of ribose/mg of protein N/hr at 30°C.

Browning of the stored samples was determined using a Beckman reflectancy unit at 430 mμ. Freeze-dried samples which had been stored at −30°C were used as controls (100% reflectancy). Samples were allowed to "reconstitute" in a slight excess of water and 3-cm diameter pieces were

punched from each. These were transferred to 3 × 0.6-cm aluminum dishes before determining the reflectancy.

RESULTS

Effect of Freeze Drying on Glucose and Ribose in Lingcod Muscle

Ten portions of lingcod muscle were blended and extracted with perchloric acid before freeze drying, and a similar number was freeze-dried before extraction. It was found that the ribose content was 587 μg/g before, and 530 μg/g after, freeze-drying. The comparable values for glucose were 530 and 735 μg/g. No explanation is offered for this increase in glucose content. These results show that freeze-drying under the experimental conditions did not reduce either the free ribose or glucose content of the muscle.

Browning of Freeze-Dried Lingcod at 25°C with and Without Added Ribose

Portions of lingcod, to some of which 1 ml of water containing 5 mg of ribose had been added, were freeze-dried and stored over H_2SO_4 at 25°C at RH values of 0, 5, and 10%. Browning was determined at intervals. Moisture determinations on 21 samples showed that there was an 81.3 ± 0.2% loss of water during freeze-drying. The results (Table I) show that discolouration proceeded at all RH values and was increased by addition of ribose. Portions of concentrated solutions from ion-exchange resin treatment equivalent to 1 g of muscle were placed along 10-cm lines on sheets of Whatman No. 40 paper, and the chromatograms were developed by the descending method in *n*-butanol:ethanol:H_2O (52:32:16) (Putnam, 1957) for 16 hr at 20°C. The dried chromatograms were sprayed with aniline hydrogen phthalate (Partridge, 1949). Marked zones corresponding to ribose and glucose appeared in those prepared from freeze-dried fish held 47 days at −30°C, while no such sugars were detectable in samples held 47 days at 25°C.

Table I. Browning of freeze-dried lingcod stored at 25°C. (Ribose (890 μg/g) and glucose (180 μg/g) were found in freeze-dried fish held at −30°C.)

Treatment	RH (%)	Reflectancy (%) after (days) 11	47	108
None	0	88	66	35
Ribose added	0	83	56	44
None	5	83	60	56
Ribose added	5	74	58	49
None	10	75	75	48
Ribose added	10	71	76	38

Effect of Added Ribose and Enzyme Treatment on Browning of Freeze-Dried Lingcod and Sole Stored at 37°C

Portions of lingcod and lemon sole muscle, to some of which were added either 2 ml of a 1-mg/ml solution of ribose or 0.8 ml of a one in ten aqueous solution of particulate enzyme, were freeze-dried and stored at 37°C over sulphuric acid at RHs over 0, 5, and 10%. Browning was determined at intervals and the results (Table II) show that it increased with time and was more rapid in samples with added ribose and slower where glucose and ribose were largely removed by enzyme treatment, especially during early storage. With the lingcod, the initial ribose and glucose contents were 400 and 75 μg/g, respectively, and 12 and 25 μg/g after 64 days. With the sole, the corresponding amounts were 290 and 31 initially, but interference in the colorometric determinations made it impossible to detect the sugars after 64 days storage. It is of interest that browning in the untreated lingcod appeared to proceed a little more rapidly at 0% RH, while with sole there was little difference between 0 and 5% RH. Paper chromatography of extracts using conditions similar to those of the preceding experiment showed a strongly positive ribose zone and a weaker glucose zone with the freeze-dried lingcod and sole stored at −30°C, but no such zones in fish stored 64 days at 0 RH.

Effect of Nitrogen Storage on Enzyme-Treated and Untreated Lingcod at 33°C

Portions of lingcod, some of which were treated with 1 ml and others with 2 ml of particulate enzyme, were freeze-dried and stored either in air or in commercial nitrogen over magnesium perchlorate at 33°C. Increase in browning was determined at intervals. Both glucose and ribose were determined before

Table II. Browning of freeze-dried lingcod and sole at 37°C after different pretreatments.

		Reflectancy (%) after (days)					
		Lingcod			Sole		
Treatment	RH (%)	7	38	64	7	38	64
None	0	57	27	22	87	31	25
Enzyme	0	63	39	27	93	54	43
Ribose	0	41	19	20	94	25	22
None	5	53	30	25	95	49	33
Enzyme	5	59	35	28	99	75	36
Ribose	5	40	28	18	45	33	18
None	10	67	34	22	74	34	41
Enzyme	10	96	35	29	91	35	45
Ribose	10	37	16	19	55	16	16

and after enzyme treatment. The results (Table III) show that removal of glucose and ribose markedly decreased the rate of browning both in air and in nitrogen, especially where over 90% of the sugars were removed. Storage in nitrogen also decreased the rate of browning considerably. However, considerable discolouration occurred after 55 days in nitrogen even in the samples where over 90% of the sugars had been removed.

DISCUSSION

Connell (1964) has emphasized the fact that "No authenticated instances have been reported of the production of freeze-dried fish which have not suffered some unacceptable degree of textural denaturation." While the present work has not involved any direct determinations of toughness, it was apparent during attempted blending of some stored samples that these were extremely fibrous. Browning reactions proceed at 0 RH a condition which will ensure complete elimination of free water in dried fish (Shewan, 1953). These reactions were decreased by enzymic removal of ribose and glucose, and even more markedly if the treated samples were stored under nitrogen. But even under optimum conditions browning occurred, perhaps due to the remaining small amounts of sugars in the fish or to traces of oxygen present in the commercial nitrogen used. In any case the results indicate the very great difficulties which would undoubtedly arise if attempts were made to prepare and store freeze-dried fish under

TABLE III. Effect of enzyme treatment on the glucose and ribose content and development of browning in lingcod stored in air and in nitrogen over magnesium perchlorate.

	Untreated	1 ml of enzyme	2 ml of enzyme
	μg/g	μg/g	μg/g
Ribose	465	79	40
Glucose	443	63	36

Treatment	Reflectancy (%) after (days)		
	16	48	55
Air stored untreated	42	25	24
Air stored + 1 ml enzyme	77	37	36
Air stored + 2 ml enzyme	84	40	41
Nitrogen stored untreated	84	46	44
Nitrogen stored + 1 ml enzyme	91	63	64
Nitrogen stored + 2 ml enzyme	98	79	67

conditions which would ensure complete absence of browning reactions. It has long been recognized that addition of sulphur dioxide, and removal of free sugars and amino acids by washing with cold water will markedly reduce browning of subsequently heated fish flesh (Tarr, 1950). Moreover certain antioxidants are known to retard development of oxidative rancidity in frozen fish (Tarr, 1947) and in oily fish meals (March *et al.*, 1962). It is possible that application of such methods may be useful in extending the keeping quality of freeze-dried fish. The browning experienced in the present investigation was accompanied by unpleasant ammoniacal and acrid odours which would tend to make products unacceptable.

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Biomicroscopic, Gross, and Microscopic Observations of Corneal Lesions in the Lake Trout, *Salvelinus namaycush*[1]

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ABSTRACT

A description has been given of a corneal lesion which occurs in hatchery-reared lake trout. The lesion is characterized by a general cloudiness of the cornea leading to marked epithelial erosion. A secondary inflammatory response with pigment migration and uveitis is observed. The condition terminates in endophthalmitis and complete loss of the functional eye and destruction of choroid, retina, lens, and iris. Although many factors may be involved, the osmotic gradients to which the freshwater teleost eye is exposed are believed to play a vital role in the development of this corneal pathology.

During the last 5 years there has been a movement underway to restock waters with lake trout which had been depleted of this species due to lamprey infestation. As a consequence both state and federal hatcheries have increased their lake-trout rearing programs. At some hatcheries operated in the State of Michigan it has become evident that hatchery-reared lake trout are prone to develop several types of eye abnormalities, among which is a severe corneal lesion. The purpose of this paper is to describe the pathogenesis of the corneal opacity as observed in hatchery-reared lake trout.

MATERIALS AND METHODS

The 2- to 8-year-old lake trout (*Salvelinus namaycush*) observed were obtained from the Michigan Conservation Department fish hatchery at Harrietta, Michigan. In the laboratory they were kept in fiberglass tanks at 10°C under conditions of 15 hr light and 9 hr darkness each day. Tissues were fixed in Dietrichs fixative (10 parts formaldehyde, 29 parts 95% ethyl alcohol, 2 parts glacial acetic acid, and 29 parts water) for 24 hr or longer and then dehydrated and cleared in T400 tetrahydrofuran, histological grade (Fisher Scientific Co., Chicago, Ill.) used according to Houst (1958). All other histological procedures used followed those given in the AFIP Manual (1960).

The biomicroscope (also referred to as "slit lamp") is an instrument which combines the binocular stereoscopic microscope with a special illuminating system which permits a very intense thin beam of light to be focused on the various layers of the *in vivo* eye. In use, the light beam is directed into the animal's eye in such a way that the observer sees the beam at an angle

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of about 30 to 40 degrees. Although the cornea appears to be entirely transparent in diffuse light the focused beam reveals that this is not necessarily true. Media like the cornea and the lens have a complex cellular structure which causes internal dispersion of light known as relucency. In the eye this phenomenon accounts for the formation of opalescent blocks of various intensities which are in the shape of rectangular parallelepiped prisms. The external and internal surfaces of the blocks are slightly curved owing to the anatomic shape of the cornea. Any corneal scar, dystrophy, or infiltration causes either an increase or decrease in the localized optical density. In summary, the slit lamp allows one to observe a 20-μ "optical section" through the anterior portion of the living eye. The composition of this section is dependent upon the optical properties of the tissue which may or may not correlate well with histological observations. In most cases a corneal lesion will be seen with a slit lamp before it shows up in histological sections. For a complete discussion of the biomicroscopy of the eye the reader is referred to Berliner (1949).

OBSERVATIONS AND DISCUSSION

When the trout reach the age of 2 years the corneal opacities first appear. At this same age the fish first become susceptible to sunburning (Allison, 1960). As trout become older, more of them develop defects of the eye, and the severity of the lesion increases. Among lake trout that are 2 years old, half have normal corneas whereas among trout that are 6 years old only 14% have normal corneas. The number of fish showing bilateral corneal opacities as compared to those fish showing unilateral opacities remains constant with age at a ratio of 1:1.

An arbitrary classification of the corneal lesions by stages has been assigned and is discussed below. The frequency of occurrence of the various stages observed in 200 randomly selected 2-year-old lake trout eyes and suggested progression of the lesion are given in Table I.

Stage 1 is characterized by keratectasia, a marked anterior protrusion of the cornea similar to human keratoconus or keratoglobus, which results in an increased anterior chamber and aqueous humor volume. The radius of curvature of the cornea may be either nearly normal or it may come to a sharp, well-defined conical apex (see Fig. 1). The corneas in this stage show no change in their transparency. Based on the chi-square statistic the incidence of keratoconus is independent of the age of the fish. The ratio of bilateral to unilateral keratectasia remains at 1:3 in both the 2-year-old and 6-year-old fish.

Stage 2 is considered a sequel to stage 1 in the pathological progression of this lesion. There are statistically more eyes showing this advanced lesion in the older fish. It is characterized by the presence of marked keratoconus-keratoglobus deformities (conical or globular protrusions) of the cornea and, in addition, pronounced changes in the optical transparency. Usually the opacity occurs initially at the apex of the keratectasia. This clouded area may remain localized or may show tendencies to spread to the more peripheral margins of the cornea.

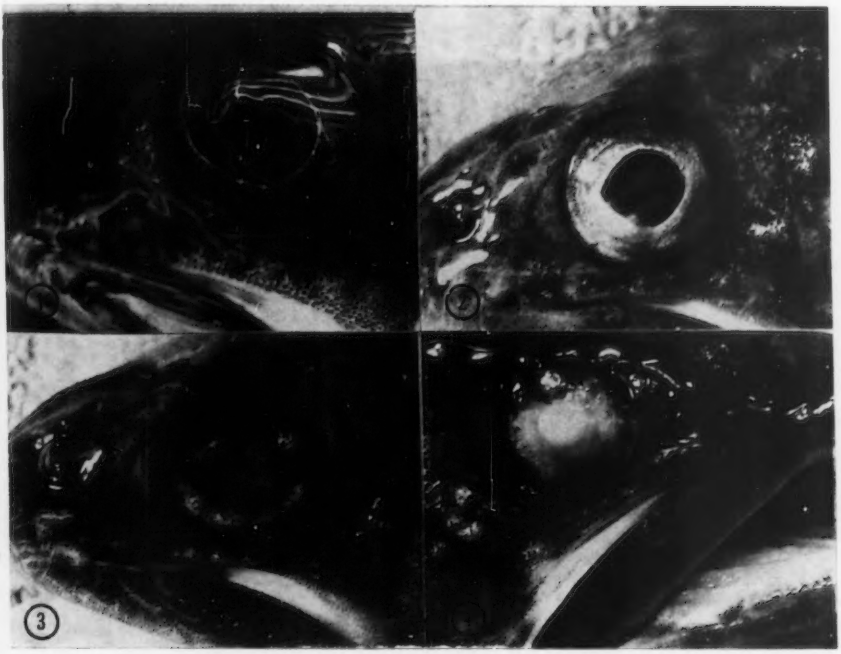


FIG. 1. Anterior corneal reflex as seen by use of Placido disc. (Interpreted like contour lines.)

FIG. 2. Localized opaque bands representative of stage 1a corneal dystrophy.

FIG. 3. Early stage 3 keratitis with marked pigment migration, diminution of keratectasia, and decreased pupil size.

FIG. 4. Similar to Fig. 3 except the lens is cataractous and keratitis more pronounced.

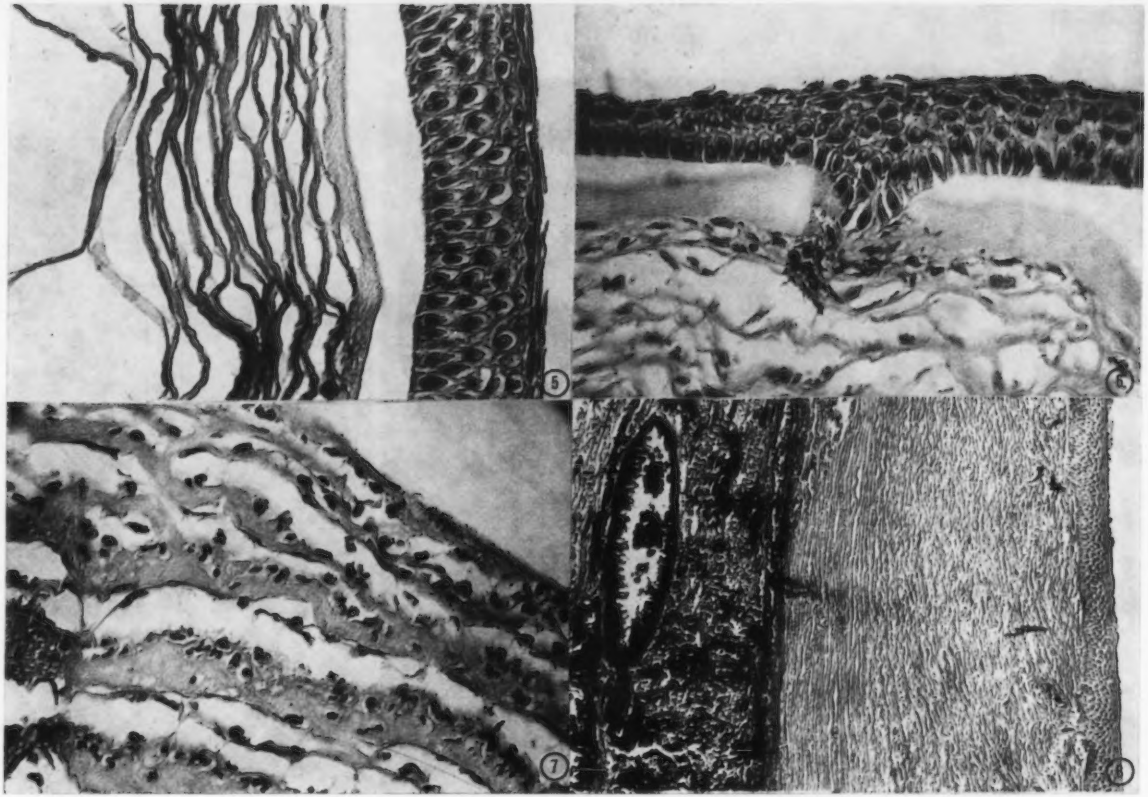


FIG. 5. Normal histology of the lake trout cornea. (× 100)

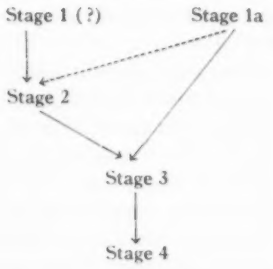
FIG. 6. Projection of epithelium through Bowman's membrane. (× 100)

FIG. 7. Lymphatic infiltration of the stroma, erosion, and thinning of corneal epithelium. (× 100)

FIG. 8. Adherent leucoma scar and healed corneal ulcer. (× 46)

TABLE I. Frequency distribution of the different stages of corneal pathology in 200 eyes from 2-year-old lake trout and suggested progression of the lesion.

Frequency distribution		
Stage	Number	Progression of lesion
Normal	129	
1	10	
1a	32	
2	15	
3	11	
4	3	



Stage 1a is believed to represent a second form in which the corneal involvement may begin. In this stage the lesion is represented by a cornea which has a normal radius of curvature and anterior chamber depth. The cornea takes on a milk cloudiness which may be (a) evenly distributed over the entire surface, (b) localized in several small discrete areas, or (c) represented by a band of opacity moving across the cornea (Fig. 2). The incidence of Stage 1a cornea dystrophy is independent of the age of the fish. As indicated in Table I the progression from stage 1a is normally to stage 3, however, some eyes may progress from stage 1a to stage 2 as indicated by the broken arrow.

It is believed that commencing with the onset of corneal clouding the lesion is irreversible and progresses to its termination. Various degrees of ulcerative keratitis characterizes stages 3 and 4. In stage 3 one sees the beginning of a marked softening of the epithelium and epithelial erosion (Fig. 3). Marked migration of pigment can be observed at the peripheral margins of the cornea. The iris becomes hyperemic and the pupil is much smaller than normal, probably indicating some degree of inflammation (uveitis). With the disruption of the protective barriers (epithelium and Bowman's membrane) by the progressive keratitis, we believe that the fluid contained in the anterior chamber of the eye seeps through the cornea resulting in a diminution of the keratectasia.

Stage 4 is characterized by the total perforation of the central portion of the cornea. The perforation eventually heals over with fibrotic tissue and the result is a completely nonfunctional structure (Fig. 4). At the time of ulceration a phacocele (dislocation of the lens) forms in response to a decreased intra-ocular pressure. The lens may be completely prolapsed through the ulcer or it may become cataractous and undergo absorption. There is complete loss

FIG. 8. Adherent leucoma scar and healed corneal ulcer. (× 46)

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of the aqueous humor resulting in a prolapse of the iris and formation of an adhesion of the iris to the cornea (anterior synechia). At no time during the progression of this lesion does one observe capillary or neural injection of the corneal tissue. Older fish were observed to have a much higher frequency of the more advanced stages.

Biomicroscopy and histological examination of mammalian corneas has resulted in the identification of five distinct anatomical structures: epithelium, Bowman's membrane, stroma, Descemet's membrane, and endothelium. Histologically, the teleost cornea is composed of an epithelium, Bowman's membrane, and a stromal layer with no readily discernible Descemet's membrane or well-developed endothelium (Smelser, 1962). The optical properties of intact, viable corneas which we observed provided additional evidence that Descemet's membrane and the endothelium are poorly developed structures in lake trout eyes. In other respects, the histological and biomicroscopic observations of the teleost and mammalian eyes are comparable. Slit lamp and histological findings on corneas from stage 1 eyes show no abnormalities with the exception of the increased depth of the anterior chamber and possible thinning of the central area of the cornea (Fig. 5). The separation of the epithelium from the stroma and the separation of the stromal fibers is a sectioning artifact found in normal tissue and are not observable with the slit lamp. In advanced lesions the epithelium adheres tightly to Bowman's membrane. Stage 1a eyes exhibit a "ground glass" appearance which results from an increase in the optical density of the epithelial layer. In certain areas of the cornea more marked opacities are observed which begin to show signs of localized erosion. Histologically, these corneas show a disorganization of the normally cuboidal arranged basement epithelial cells. In a more advanced stage 1a and 2 eyes there are degenerative changes in the epithelium including vacuolization, pyknotic nuclei, erosion of the superficial epithelium, hyperplasia, and necrosis. There is usually no indication of an inflammatory response of lymphatic infiltration in this stage. Also, there is no indication of vascular injection of the cornea as would normally be associated with this type of lesion in mammalian species.

In examining eyes from stage 3, one observes prominent erosion and thinning of the corneal ,epithelium, an irregular thickening of Bowman's membrane and increased optical density of the stroma. There is some increase in the relucency of the aqueous humour. Pigment migration from the peripheral margin towards the central area is noticeable. Histologically these corneas show marked localized hyperplasia of the epithelium with finger-like projections working through and separating fragments of Bowman's membrane (Fig. 6). At this stage the epithelium has become more disorganized corresponding to the degree of opacity. Immediately below Bowman's membrane one finds a localized area of lymphatic infiltration. This area also contains large macrophages which are phagocytizing melanin and are probably responsible for much of the pigment migration observed (Fig. 7). There are indications of uveitis and a collection of pus containing phagocytes including neutrophilic leukocytes and lymphocytes in the anterior chamber (hypopyon).

In the stages characterized by ulceration we also have a corresponding acute inflammation caused by secondary bacterial or virus infection. Figure 8 shows a cornea with marked stromal and epithelial erosion having almost reached the point of rupture. At this time the permeability of the cornea is such that aqueous humour may seep out of the eye due to a positive intraocular pressure. Slit lamp observations on these advanced stages show pyramidal cataracts resulting after the lens has lain for some time against the perforated central ulcer of the cornea. In the more advanced stages corneal opacity is so severe that slit lamp observations are impossible.

The typical hypopyon found in eyes of mammals consists of an accumulation of leukocytes in the bottom of the anterior chamber. At no time in the progression of the lesion of fish is a typical hypopyon observed as a compact mass of sedimented cells but instead, the cells seem to remain suspended in the very viscous aqueous humour. The major source of these white cells is probably the hyperemic vascular supply of the iris. At the time of perforation, the acute inflammation develops into endophthalmitis characteristic of the terminal stage 4. Histologically, the final stage of this disease is represented by the forming of an adherent leucoma scar and ultimately the healing of the ulcerated area by hyperplasia of the epithelium and fibrotic infiltration of newly formed keratoblasts. In old scars the keratoblasts become keratocytes with intercellular fibrillae much like normal corneal stroma. Anatomically one sure criterion for recognizing a scar is a defect in Bowman's membrane because this structure does not have the ability to regenerate or knit together. Prior to stage 4 the lens has either been extruded from the eye through the ulcer or it has undergone absorption. At this stage the retina has undergone complete destruction and the ocular cavity is filled with fibrotic tissue and lymphocytes and shows extensive pigmentation. The optic nerve remains exceptionally free of any pathological changes and there is no indication of migration of this inflammatory response out of the immediate confinement of the scleral coat. Some degeneration of the ocular muscles is noted.

In the mammalian species recovery from corneal lacerations, abrasions, and dystrophy is usually very rapid owing in a large degree to the fact that isosmotic fluids bathe both sides of the cornea. In freshwater teleosts, the corneal epithelium is bathed by a very hypoosmotic fluid. The epithelium and Bowman's membrane of the teleost must play a major role in maintaining a structure relatively impermeable to the osmotic influx of water (Edelhauser *et al.*, 1965). Any physical or chemical insult to the integrity of Bowman's membrane or the epithelium would result in an irreversible "chain-reaction-like" influx of water leading ultimately to total ulceration and complete corneal opacity. Possibly because of differences in osmotic gradients, and other factors, we find no parallel ocular pathology described in mammalian ophthalmology. The initial insult resulting in permeability changes is believed to occur in the epithelium. The possibility that this is the result of some type of metabolic insufficiency of the epithelial cells is being investigated. Genetic involvement has been ruled out because this lesion has been observed in a wide variety of

brood stock. Preliminary information from hatchery studies indicates possible aggravation of this corneal condition by exposure of fish to ultraviolet and visible radiation.

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Zone Electrophoretic Comparison of Muscle Myogens and Blood Proteins of Artificial Hybrids of Salmonidae with Their Parental Species[1]

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ABSTRACT

Starch-gel zone electropherograms of the muscle myogen and blood hemoglobins and disc electropherograms of the serum proteins of a number of artificial hybrids involving the *Salmo*, *Salvelinus*, and *Cristivomer* genera are compared to their respective parental species. The pattern variations observed in the serum proteins and muscle myogens in many of the parental species are discussed in relation to the frequent variations obtained in F_1-hybrid patterns. The necessity is pointed out for defining biochemical as well as morphological uniformity of parent species used for artificial hybridization studies, particularly as freshwater species of fishes, in contrast to marine species, frequently hybridize in nature or have hybridized in recent history, judging from the variations in their protein patterns. The extent of known hybridizations in nature, which are indistinguishable morphologically but are frequently manifested at the molecular level as variations in protein patterns, and the usefulness of biochemical techniques in spotting these breakdowns of species lines, are discussed.

INTRODUCTION

A morphometric study of about one million specimens each of both freshwater and marine fishes has led Hubbs (1955) to two general conclusions on the subject of hybridization in nature: (a) the frequency of hybridization is largely a function of the environment, and (b) hybrids are excessively rare among marine fishes in contrast to northern freshwater fishes. Hubbs prepared charts of six families (Catostomidae, Cyprinidae, Cyprinodontidae, Poeciliidae, Percidae, Centrarchidae) of freshwater fishes showing species which do not co-habitate, and those that do and have produced natural hybrids. The few authenticated cases of hybridization in marine fishes, as in the Pleuronectidae (Hubbs and Kuronuma, 1941) have also been described in the same article. Frequently, new species described have upon further examination resolved into hybrids of known species as in the case of *Lepomis euryorus* McKay which proved to be a cross between the green sunfish, *Lepomis cyanellus* Raf., and the pumpkinseed sunfish, *L. gibbosus* (L) (Hubbs and Hubbs, 1931). Similarly *L. ischyrus* was established as a hybrid of the bluegill, *L. macrochirus* Raf., and the green sunfish (Hubbs and Hubbs, 1932).

The first detailed accounts of artificial hybridization experiments of Salmonidae were reported by Day (1884, 1886, 1887) who succeeded in crossing *Salmo salar* and *Salmo trutta*, attaining 25–30% fertility. A detailed review of

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interspecific and intergeneric hybridization experiments with Salmonidae carried out in Europe and in North America has been included in an article by Alm (1955) describing the contributions of the Fishery Board of Sweden in these areas of investigation. The most successful of these artificially produced fertile hybrids appears to be the cross between the female lake trout (*Cristivomer namaycush*) and the male eastern brook trout (*Salvelinus fontinalis*) (Buss and Wright, 1956; Stenton, 1950, 1952) termed the "splake," a name suggested by the biologists of the Ontario Department of Lands and Forests. The fact that each of these species, as well as the splake, have chromosome counts of 84 (Svardson, 1945; Wright, 1955, 1956) largely influenced egg and fry viability. The reciprocal cross, that of male lake trout and female brook trout, produced a significant number of hybrids with caudal deformity (Stenton, 1950, 1952; Buss and Wright, 1956) indicating that egg size is another one of many factors that determine successful hybridization. Most other crosses and reciprocal crosses of the members of the Salmonidae family, the brown, brook, rainbow, and lake trouts and the landlocked salmon, led to varying degrees of infertility of the eggs, deformed frys, and sterile offsprings (Buss and Wright, 1956). Of 25 taxonomic measurements and counts on four specimens of splake, nine were intermediate, six resembled the brook trout, and 10 the lake trout. True intermediacy is not necessarily the rule in F_1 hybrids.

The application of biochemical systematics (Alston and Turner, 1963) in recent years has provided further means of investigating biochemical uniformity within species (Tsuyuki and Roberts, 1963; Tsuyuki *et al.*, 1965a, b). Starch-gel and polyacrylamide zone electrophoretic techniques were found to be particularly convenient and useful in this respect as the group of substances under investigation, the proteins, are especially well suited to these types of analyses as well as to serological approaches. It is common knowledge today that within the structure of the protein molecule is to be found much of the genetic information useful for phylogenetic investigations. The results from these studies have generally proved to be complementary to morphometric investigations. Once it has been established that electrophoretic patterns of muscle myogens and blood proteins are invariable species-specific characteristics of living organisms, these approaches can be readily applied to problems in speciation and hybridization. This has been demonstrated (Tsuyuki *et al.*, 1965b) in a study of large numbers of *Oncorhynchus nerka* from a number of spawning grounds and in a variety of physiological conditions. A less extensive study has been carried out in four other species of this genus. With some exceptions, 39 marine species commonly available in the North Pacific coastal waters of North America displayed similar degrees of constancy and species specificity in their muscle myogen and blood protein patterns (Tsuyuki *et al.*, 1965a). The constancy of these biochemical characteristics in marine and anadromous species appears to be in agreement with one of the general conclusions of Hubbs (1955) that hybridization is excessively rare in marine species. One clear-cut exception, obtained with samples from two successive years, is the systematic pattern variation of the sablefish (Tsuyuki *et al.*, 1965a) from Alaskan waters suggesting natural hybridization.

Further examples of hybridization of species will no doubt come to light by the application of biochemical approaches to those hybrids which resemble their parent species so closely that morphometric characteristics become indistinguishable.

In striking contrast to marine species, freshwater species from the Great Lakes areas and in local waters, and including the freshwater *Salmo*, show some variations in protein patterns. In some species, polyacrylamide serum protein patterns have been found to vary in as many specimens as investigated, again in agreement with the conclusions of Hubbs, and suggesting further that present-day representatives of these species must have gone through a period of extensive hybridization with closely related species, the whole series of events manifesting itself as variations in protein pattern under the closer scrutiny of biochemical techniques. It is therefore of considerable importance that artificial hybridization studies be carried out with the knowledge that parent species be biochemically as well as morphologically uniform. The use of the Pacific salmon in hybridization studies, as has been carried out by Foerster (1935), meets both these qualifications as these anadromous species have been stabilized for longer periods of time in relatively constant environment, and would therefore provide hybridization results with the assurance that the parent species are as uniform as modern techniques can demonstrate.

In the present communication muscle myogens and blood proteins of artificial hybrids and their parent species of some freshwater members of Salmonidae are examined by starch-gel and polyacrylamide disc electrophoresis.

MATERIALS AND METHODS

The parent species of brown trout (*Salmo trutta*), speckled trout (*Salvelinus fontinalis*), alpine trout (char) (*Salvelinus alpinus*), lake trout (*Cristivomer namaycush*), rainbow trout (*Salmo gairdnerii*), and all the artificial hybrids formed from some of these species were obtained, frozen in dry ice, from the Laboratory for Experimental Limnology,[2] Maple, Ontario. The hemoglobin and serum protein from the above samples were obtained by a previously described method (Tsuyuki *et al.*, 1965a; Vanstone *et al.*, 1965) and air-expressed to this laboratory in vacuum bottles containing ice. In all the crosses, the parent species mentioned first represents the female specimen except in the brown × speckled where this order was reversed.

Starch-gel zone electrophoresis was carried out as described in previous communications (Tsuyuki, 1963; Tsuyuki *et al.*, 1965a). A Canalco Model 12 disc electrophoresis apparatus was employed for the polyacrylamide separation of serum protein samples. The pre-mixed standard gel appeared to give sufficient resolution and was used according to the instructions provided, with minor variations. In this case, 2.5 ma was used for each column for a period of 1½ hr, as the rate of migration of fish sera was a little slower than mammalian, but

[2]The Laboratory for Experimental Limnology, Maple, Ontario, is jointly operated by the Research Branch, Ontario Department of Lands and Forests, and the Department of Zoology, University of Toronto.

chiefly because there was some heating of the column at 5 ma per column at room temperature.

The stained polyacrylamide as well as the starch gels were photographed by the photographic section of the Department of Extension at the University of British Columbia. To facilitate photographing the polyacrylamide gels in a vertical position, a special rack was constructed from plexiglass capable of holding two rows of 15 tubes each fastened by means of small broom clamps. The rack with the tubes in place was then hung in front of an ordinary G.E. X-ray viewer and photographed by transmitted light. All photographic reproductions were made identical in size so that a print of any polyacrylamide gel could be compared with that of another.

RESULTS AND DISCUSSION

Polyacrylamide disc electrophoretic separations of serum proteins of the parent species of Salmonidae are shown in Fig. 1. In hybridization studies it is of importance to know the biochemical uniformity of the parent species. Although only a few specimens of each species were available in the present study, disc electrophoretic separations of serum proteins of large numbers of *Oncorhynchus nerka* (Tsuyuki *et al.*, 1965b) showed a degree of uniformity which places credence on the analyses from a few individuals provided the species are uniform. The two samples labelled "brown" (Mount Pleasant stock) differ considerably from each other while the three "Wisconsin brown," though uniform within themselves, vary considerably from the first two. All three variants of speckled trout, of which two specimens each were available, though comparatively uniform within each group show minor variations between groups particularly near the origin and in the case of the "speckled," in one of the major zones near the middle of the pattern. It appears, therefore, that the brown and speckled trouts show variations reflecting perhaps differences in racial stocks, diet, or the mixed origins of these stocks. Only a single sample of alpine trout (Lake Geneva stock) was available for analysis. The three specimens of lake trout appear to be relatively uniform, while one of the rainbow trout patterns varied somewhat from the remaining two. It would be interesting to investigate on a larger scale the biochemical uniformity of *Cristivomer namaycush*, which inhabits a relatively stable lacustrine habitat that differs little from lake to lake, and hence is more protected from cosmic radiation and other factors aiding evolutionary processes (Rounsefell, 1962). It is also evident that although individual differences do exist, these variations are well within the overall characteristic patterns of each species. Finally, it should be emphasized that in the absence of biochemical uniformity in the parent species employed for hybridization studies, differences in morphological and other characteristics of the F_1 hybrids produced by different investigators are to be expected. It should also be borne in mind that serum proteins may be influenced more than muscle myogens by factors such as diet. However, such variations, if any, can be readily demonstrated by sampling specimens from a stock of fish reared under the same conditions. For practicality, samples of blood could be drawn from the

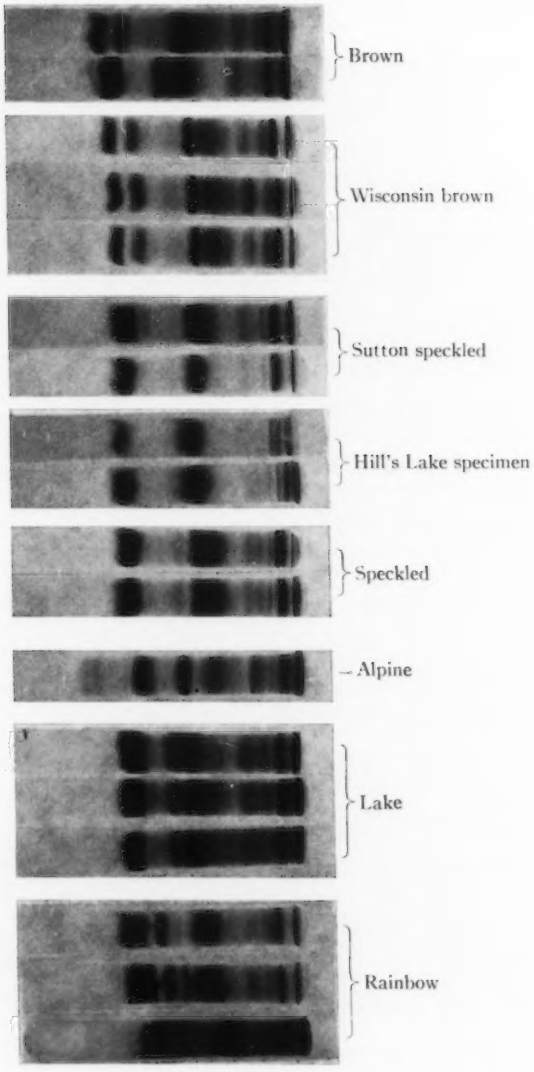

FIG. 1. Polyacrylamide disc electropherograms of the blood plasma of parental species of Salmonidae used for hybridization. The anode is to the left.

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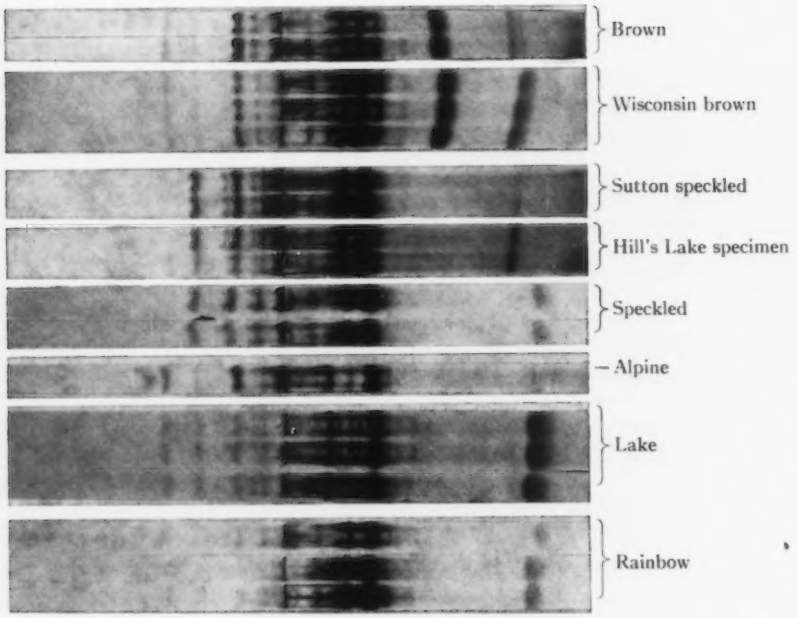

FIG. 2. Starch-gel electropherograms of the muscle myogens of Salmonidae. The anode is to the right and the cathode to the left.

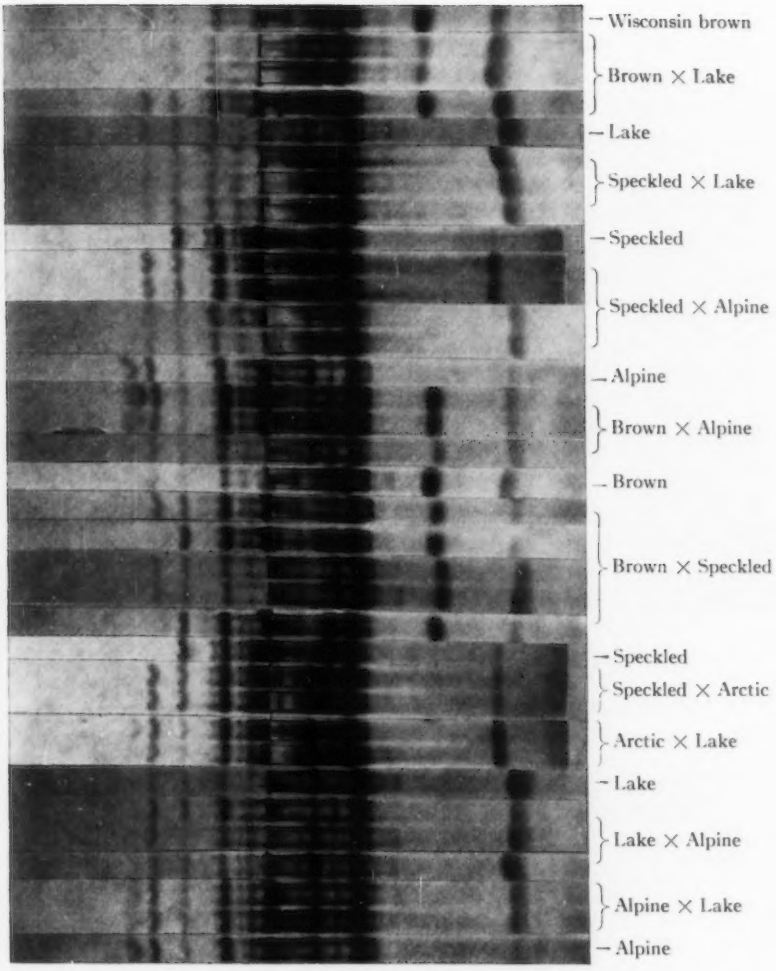

FIG. 3. Starch-gel electropherograms of muscle myogens of artificial hybrids of Salmonidae and their parental species.

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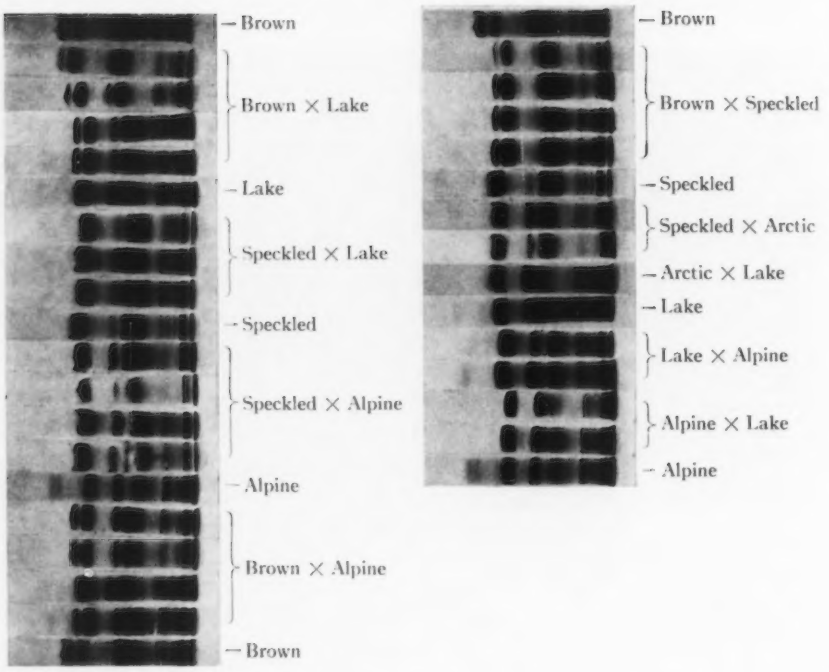

FIG. 4. Polyacrylamide disc electropherograms of the blood plasma of artificial hybrids of Salmonidae and their parental species.

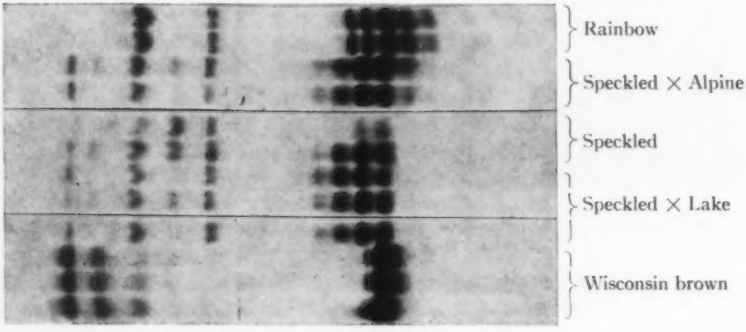

FIG. 5. Starch-gel electropherograms of the multiple hemoglobins of some hybrids of Salmonidae.

parent species and their serum protein uniformity checked before artificial hybridization.

In Fig. 2 are shown starch gel electrophoretic patterns of the muscle myogens of the same samples from which serum proteins were analysed in the previous figure. The most obvious difference in the "brown" trout is in the most anodic zone which separated into two in the Mount Pleasant stock. What appears as differences in the mobility of this zone in the various species is due to technical difficulties encountered in starch-gel electrophoresis which cause these rapidly migrating anodic zones to move out in a curvature. Subsequent editing and rearrangement of these photographs to facilitate discussions make it difficult to line up the zones properly.

Thus the variations observed in the serum patterns are reproduced in the muscle myogen patterns. Of the three groups of speckled trout, that labelled simply "speckled" shows only a single zone in the region of the next-to-the-most-cathodic zone, while one of the Hill's Lake speckled has an extra zone at a position most cathodic from the origin. The lake trout again show uniformity of pattern as was also the case with rainbow trout. Species specificity is maintained throughout. Of special interest is the characteristically strong anodic zone of the brown trout which is absent in the other species and present in all F_1 hybrids possessing this species as one of their parents.

The muscle myogen patterns of hybrids are presented in Fig. 3 along with their respective parent species. A close examination of the patterns of brown × lake hybrids shows that the first two (beginning from the top) are more closely allied to the lake trout, especially in the relative intensity of the single anodic zone peculiar to the brown trout. The third resembles brown trout in relation to that same zone. In all cases, however, all the zones present in both parent species are represented in the hybrid to a greater or, usually, lesser degree. The relative intensity of the third cathodic zone (from origin) of the third hybrid may well be a reflection of the difference in intensity of this same zone in the two strains of brown trout discussed in Fig. 2.

The three specimens of splake (Fig. 3) appear to show uniformity in patterns. The patterns on the cathode side, however, suggest that the speckled trout used for these hybrids was not of that shown but rather those represented as "speckled" in Fig. 2. A similar explanation appears applicable to the next hybrid, speckled × alpine. Of the three specimens of brown × alpine the first two are associated more with the Mount Pleasant stock (Fig. 2). All three, however, are definitely hybridized because of the presence of the characteristic brown trout anodic zone. Of the five brown × speckled specimens, the first, second, and fifth show more speckled characteristics than third and fourth. In the absence of the parent arctic species it is not possible to discuss the speckled × arctic and arctic × lake hybrids. In the lake × alpine and its reciprocal crosses, the first, fourth, and fifth are closer to alpine. As only a single parental specimen of alpine trout was available, the patterns shown in Fig. 3 and 4 are reproductions of the same individual.

Disc electrophoretic serum protein patterns of the corresponding myogen patterns in the previous figure are shown in Fig. 4. Approximately the same points of discussion, such as the dominance of one or the other species and speculation as to what parent strains were employed, brought out in the previous figure apply to the serum protein patterns.

With the possible exception of a few very faint zones, the serum protein and muscle myogen patterns of all the hybrids examined do not provide evidence for the formation of any new hybrid proteins which is in agreement with the findings in the various animal hybrids reviewed by Fox *et al.* (1961). Exceptions are to be found in esterase proteins (Schwartz, 1962) of inbred lines of maize, alkaline phosphatases (Levinthal *et al.*, 1962) in intergeneric bacterial hybrids and, more important to the present studies, in the hemoglobins produced in the artificial hybrids of the bluegill (*Lepomis macrochirus*) × warmouth sunfish (*Chaenobryttus gulosus*) and the green sunfish (*Lepomis cyanellus*) × warmouth sunfish (Manwell *et al.*, 1963). Oxygen equilibria of hybrid hemoglobins displayed greater heme–heme interactions than those of either parental species, resulting in superior oxygen unloading qualities, and coincided with the increased rate of growth and vigor shown by these hybrids.

It is unfortunate that the hemoglobins of the lake trout used in these hybridization experiments were not available to compare with those of the splake (Fig. 5), the most successful of these hybrids and which also show some of the hybrid vigor phenomenon. The speckled × alpine was the only other hybrid whose hemoglobins were analysed but again, the blood of one of the parental species, the alpine, was not available for comparison. In Fig. 5, the considerable deterioration of the hemoglobin sample from the first speckled trout probably reflects the differences in patterns between the two specimens. The relative dominance of one or the other parental species is again reflected in the differences in the individual patterns within each of the two hybrids. Species specificity in their multiple hemoglobin patterns is again maintained for the rainbow, speckled, and brown trouts.

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Post-Spawning Death of Pacific Salmon: Sockeye Salmon (*Oncorhynchus nerka*) Maturing and Spawning in Captivity[1,2]

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ABSTRACT

It has been shown that sockeye salmon, *Oncorhynchus nerka*, will start to eat soon after capture during their freshwater spawning migration. Some will continue to eat up to, during, and after spawning, while others stop eating shortly before spawning. The effect of feeding has been investigated histologically in a number of tissues by comparison of feeding fish, unfed controls, and fish spawned in the wild. Atrophy, and often degeneration of the liver, stomach, and intestine in post-spawned unfed controls and fish spawned in the wild was equally marked, but was absent or much less pronounced in fed fish.

INTRODUCTION

All species of Pacific salmon, genus *Oncorhynchus*, die shortly after their first spawning (Clemens and Wilby, 1961) except for certain precociously maturing male spring (chinook) salmon, *O. tshawytscha* (Robertson, 1957; Gebhards, 1960). This phenomenon, being relatively rare amongst vertebrates (Robertson, 1957) has been investigated by both physiological (Robertson *et al.*, 1961) and histological (Robertson and Wexler, 1962) techniques using fish spawning in the wild. However, in their natural environment Pacific salmon are subjected to several environmental and physiological changes and unaccustomed stresses among which may be listed (i) movement from salt to fresh water, (ii) often, but not always, lengthy freshwater migrations, (iii) bacterial and fungal infections, (iv) increased water temperatures, (v) starvation, and (vi) sexual maturation. The contribution of each of these factors to changes in the characteristics of the salmon and to its eventual death is difficult, if not impossible, to distinguish under natural conditions. Additionally, the process of capturing a fish in the wild could induce rapid physiological changes which might mask the true physiological conditions of the animal just prior to capture (e.g. Leloup-Hatey, 1958). In an effort to control a number of these factors and, as a consequence, to obtain a clearer picture of the changes leading to post-spawning death in Pacific salmon, we have initiated the present study on sockeye salmon, *O. nerka*. One particularly important aspect of the investigation has been facilitated by the earlier finding that migrating sockeye salmon would resume feeding in fresh water some 4 months after captivity (McBride *et al.*, 1963) although it was not known if they would do so earlier. This paper

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records the culturing techniques employed and some preliminary results on the effects of feeding during maturation and spawning, including the results of histological studies of the stomach, intestine, and liver of maturing fish.

MATERIALS AND METHODS

Fish

Sixty-four sockeye salmon were captured during June 1963 as they entered Great Central Lake, Vancouver Island, B.C., on their spawning migration (Idler *et al.*, 1961). Methods of capture of fish and their transportation to the laboratory and control of fungal and bacterial infections have been described (McBride *et al.*, 1963). One week after capture the fish were divided into two groups of 48 and 16 fish and each fish was tagged for identification. The first group was fed a diet of frozen marine zooplankton (see below). The fish were weighed monthly until spawning, after which weighing was discontinued to reduce handling. When the fish appeared to be ready for spawning they were transferred, one pair at a time, to spawning tanks containing gravel. When the female had finished spawning, the pair was placed in a tank containing only post-spawners, fed and unfed fish again being kept separately.

Food

Marine zooplankton, predominantly *Euphausia pacifica*, were collected from the scattering layer in Saanich Inlet, Vancouver Island (Bary *et al.*, 1962) and stored at −30°C. Partially frozen 1-cm cubes of these zooplankton were presented to the fish once daily, the feeding being continued until the fish appeared to be satiated.

Histological Techniques

Fish were immobilized by severing the spinal cord just caudal to the head, and tissues were immediately excised and placed in Bouin's fixative. Following dehydration and clearing, the tissues were embedded in Paraplast and sectioned at a thickness of between 4 and 6 μ. Tissues on which fat examinations were to be made were fixed in calcium formal, embedded in gelatin, and then sectioned to a thickness of 10 or 16 μ with the use of a cryostat. Sections were stained with Mayer's hematoxylin and eosin for general structural examination and with Masson's triple stain, Heidnhein's azan, and Schiff's periodic acid for more detailed cytological examinations. Sudan Black B was used as the fat stain.

Samples of stomach, both pyloric and cardiac portion, intestine, and liver were taken from 16 sexually undeveloped "sea-green" adult fish, and 12 fish spawned in captivity (9 fed, 3 unfed). To determine if any differences exist between fish spawned in captivity and in their natural habitat, samples of these tissues were also obtained from eight spent or spawning fish at the mouth of Lindsay Creek, Great Central Lake, Vancouver Island, during late October 1963. In addition, a few samples of the great lateral muscle were obtained from

adult "sea-green," and fed and unfed spent fish. Other tissues and blood samples were obtained from a number of fish in each of the groups for future investigation.

RESULTS

Feeding Behaviour

Few fish of the group offered zooplankton were seen to feed during the first 2 weeks of captivity. However, by the end of 6 weeks about 60% were feeding and after 2 months virtually all the fish were feeding daily. Each fish (1.5–2.5 kg in weight) ate 10–25 g of plankton per day. During October, the fourth month of freshwater confinement, it was noted that several of these fish either ceased to feed or fed only occasionally. Some of these were obviously diseased while others appeared to be healthy. These fish, one or two of which successfully completed spawning, seldom if ever resumed their earlier intensity of feeding. Of the remaining fish, the males continued to eat during spawning, although the amount of food consumed varied greatly. On the other hand, the females with one or two exceptions ate only intermittently at this time. The feeding habits of the spent fish followed one of two general patterns. Some continued to eat, albeit sparingly, while others showed a sharp increase in the amount of food consumed. One constant observation throughout this study was the cessation of feeding fairly soon after a fish was either injured or became diseased.

Changes in the Total Body Weight of Fed and Unfed Adult Sockeye During Gonad Development

Of the 39 fish[3] offered food daily from the time of capture (June) to the commencement of spawning (November) only six showed an increase in body weight during the first month, the remainder all showed a slight loss. By the end of the third month, however, only three fish were still losing weight, all the others had either attained or exceeded their original weight. Between the third and fourth month two of the three fish which had demonstrated a continual loss from the time of capture continued to do so while the third showed no change. Eight of the other fish exhibited little or no change in weight, seven lost from 1 to 5%, while 21 gained slightly. By the end of the fourth month 12 fish (10 of them males) exceeded their original weight by at least 10%.

All of the 15 unfed fish[4] lost weight during the first and second months of captivity. The weight loss of each fish during this period varied between 2 and 9% with the majority (11) losing between 6 and 9%. During the fourth month, eight fish (four males) exhibited a modest increase in body weight. Only one of these fish, however, reached its original body weight, the others regaining approximately one-half of that lost during the preceding 3 months. The remaining seven fish (two males) continued to lose weight. The losses were slight,

[3]The remaining nine fish in this group were removed either for tissue samples or because they were diseased.

[4]The sixteenth fish in this group died from unknown causes during the second month.

however. The gain in weight by nonfeeding fish was in accord with the findings of Idler and Tsuyuki (1958) regarding weight changes in sockeye salmon during the later stages of their natural spawning migration. Several fish, feeding and nonfeeding, were killed and examined. In both groups extensive degeneration of the skeletal muscle was obvious, with no detectable difference between fish from the two groups in the degree of degeneration (Fig. 1). The dry weight of the muscle in both groups had decreased to between 9 and 13% of the wet weight in contrast to an initial value of 30% in sea-run fish. It thus appeared that the feeding had not exerted any beneficial effect on the skeletal muscle. Presumably the gain in weight in the unfed, and at least partly in the fed, was the result of an uptake of water.

Spawning

Visual observation of the Great Central Lake spawning areas including the outlet of Lindsay Creek indicates that a few sockeye commence spawning during early October, with the majority doing so in a 3- to 4-week period between mid October and early November (Mr F. Boyd, Department of Fisheries of Canada, personal communication). A few fish have been seen spawning, however, as late as mid December. Spawning of the fish held in captivity started during early November 1963 and continued till early March 1964. All but five fish, one male of which acted as a mate for two females, completed spawning by the end of December. This extended spawning period was necessitated by a lack of sufficient spawning area. While the time for each pair of fish to complete spawning was generally from 4 to 6 days, those fish which had been delayed often finished in 1–3 days. A total of 28 fed (13 males) and eight unfed fish (four males) were successfully spawned. The remaining unspawned fish either died apparently as a result of disease which appeared after they reached sexual maturity or were

Table I. Influence of feeding on post-spawning survival of sockeye salmon (*O. nerka*).

Weeks following spawning	No. of fish surviving	
	Unfed	Fed
0–2	5	17
2–4	–	15
4–6	–	13
6–8	–	5
8–10	–	2
10–12	–	1
13	–	–

FIG. 1–6

EXPLANATION OF FIGURES

FIG. 1. Cross section of lateral muscle from (A) "sea-green" sexually undeveloped adult sockeye; (B) actively feeding spent female sockeye. Sections H. and E. × 120. Muscle fibers of the feeding spent fish show extensive loss of fibrils.

FIG. 2. Cross sections of cardiac stomach (A) adult male "sea-green" sexually undeveloped sockeye; (B) actively feeding spent female sockeye; and (C) male sockeye spawned in its natural habitat. All sections H. and E. × 48. Note flattening of villi and atrophy of cardiac glands in Fig. 2C, also, reduction in diameter of external muscle layers in Fig. 2B and C.

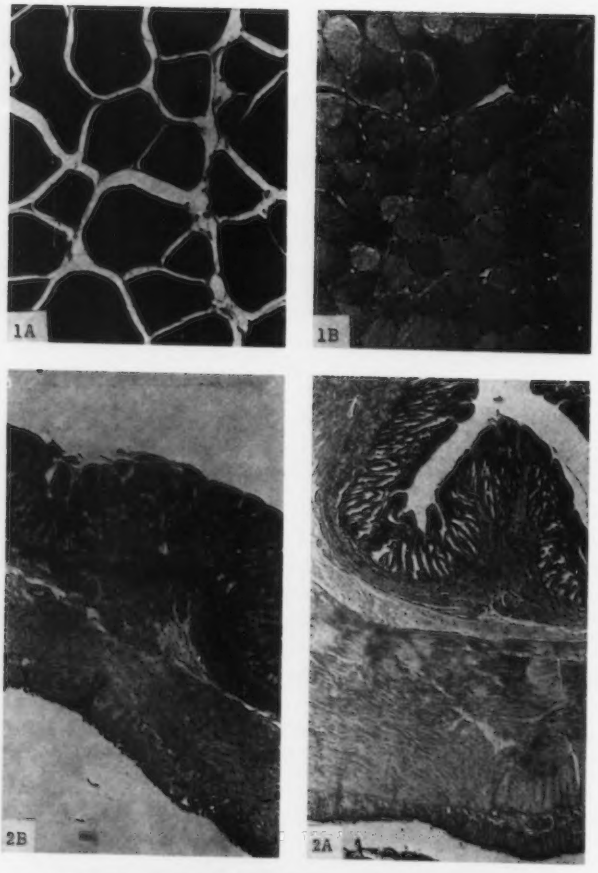
FIG. 3. Cross sections of pyloric stomach (A) adult male "sea-green" sexually undeveloped sockeye; (B) actively feeding spawned male sockeye; and (C) male sockeye spawned in its natural habitat. All sections H. and E. × 20. The unfed spawned fish (Fig. 3C) shows a marked reduction in the number of villi and in the diameter of the external muscle coat.

FIG. 4. Cross sections of intestine (A) adult "sea-green" sexually undeveloped sockeye; (B) actively feeding spent female sockeye; and (C) male sockeye spawned in its natural habitat. All sections H. and E. × 48. Note increase in external longitudinal muscle area in Fig. 4B and C.

FIG. 5. Higher magnification of epithelial layer shown in 4A and 4B. Sections H. and E. × 480. Epithelial cells in 5B (spent actively feeding female) normal in appearance.

FIG. 6. Liver of (A) adult "sea-green" sexually undeveloped sockeye; (B) unfed male sockeye spawned in captivity; and (C) actively feeding spent female sockeye. All sections H. and E. × 750. The numerous round holes throughout the cytoplasm in (A) represent dissolved fat droplets the presence of which was confirmed with Sudan black B stain. Note presence of pycnotic nuclei and cytoplasmic disintegration in (B). The cells appear normal in (C) except for shrinkage of cytoplasm.

Fig. 1–6 follow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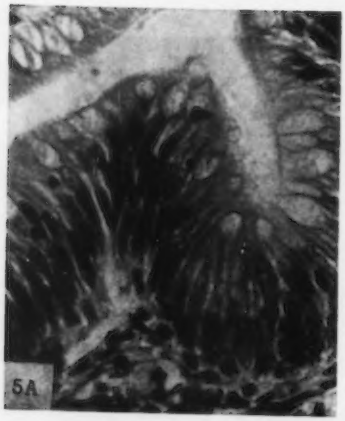
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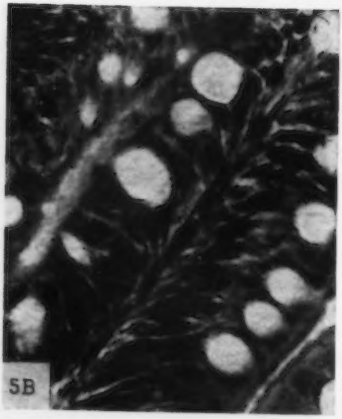
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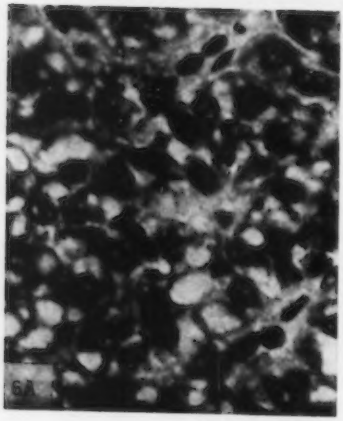
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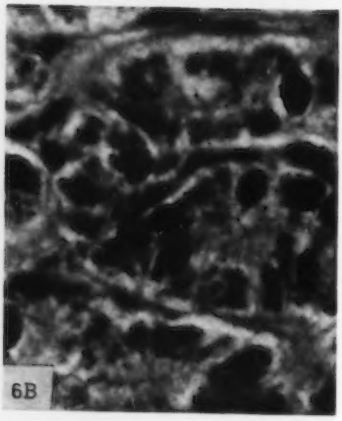
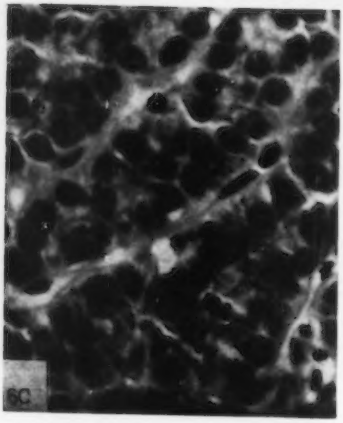

6B


6C

removed for sampling. Nine of the fed and three of the unfed fish were killed at different intervals following spawning for tissue samples. The survival periods of the remaining spent fish are given in Table I. While virtually all of these fish appeared healthy during and for a period following spawning, they all displayed obvious signs of disease at the time of death. Following spawning several striking differences were noted between the two groups of spent fish. The unfed fish were listless, generally moving only when frightened or forced to do so, and several appeared unable to maintain their equilibrium. On the other hand, the feeding fish were active and had no difficulty in maintaining their equilibrium. However, once diseased, the fed fish deteriorated rapidly and resembled the unfed in outward appearance within a few days.

Histological Changes

Stomach. The stomach of those fish (three males, four females) which fed actively before, during, and following spawning showed only minor variations from the adult sea-run sockeye (Fig. 2 and 3). There was no reduction in the size of the villi or of the epithelial layer. In four of the fish, however, vacuoles at the tip of the cardiac epithelial cells were occasionally seen.[5] In a few fish atrophy of the external circular muscle and an increase in the external longitudinal muscle were observed. These changes in the two external muscle layers were only slight and always more prevalent and pronounced in the cardiac portion of the stomach. The cardiac glands displayed no evidence of degranulation or deterioration. A variable increase in the number of eosinophilic granular cells of the mucosa, the majority of which were heavily granulated, was noted in all of the fish examined.[6]

The stomachs of two spent females which fed actively prior to spawning but either ceased to feed or fed only occasionally, showed areas of increased atrophy and signs of degeneration. The villi were reduced in size and in one were flattened. The epithelial cells were atrophied and in some instances vacuoles were seen at the tip of the cells. Extensive degranulation of the cardiac glands was visible but degeneration, when it existed, was focal. The external muscle in both the cardiac and pyloric areas of these two fish were characterized by a noticeable decrease in the circular muscle accompanied by an extensive increase in the longitudinal muscle. The presence of newly deposited collagenous tissue was detected in the tunica propria, the thickened stratum compactum, and occasionally in the external longitudinal muscle. No apparent differences in the eosinophilic granular cells were noted between these fish and those which continued to eat throughout and following spawning.

Contraction of the stomach was observed in all the unfed, spawned fish examined. No consistent differences were evident between the tissues or cells

[5]Greene (1913) described a similar finding in starved immature salmon, force-fed with olive oil, and identified the vacuoles as fat droplets. Fat stains were not carried out with this tissue in the present study.

[6]This increase during gonad development was also observed by Greene (1913) and Robertson and Wexler (1960) in their studies of migrating adult Pacific salmon. The function of these cells remains obscure.

of the stomach from unfed fish spawned in captivity (two females, one male) and from those spawned in their natural habitat (two females, six males). The villi were visibly shrunken and occasionally flattened. The epithelial cells were for the most part low columnar but did not appear to be disintegrating. Degranulation of the cardiac glands was a constant finding and in a few fish degeneration of the cells was also observed. Considerable new collagenous tissue was evident throughout the mucosa and in the greatly enlarged longitudinal muscle. The external circular muscle was visibly atrophied and areas of focal degeneration were not uncommon. While a definite increase in the number of eosinophilic granular cells was seen, a considerable variation was evident in both their number and in their degree of granulation. In a few instances the cells were partially or totally degranulated while in others they were heavily granulated.

INTESTINE. In general, those changes observed in the stomachs of the fed and unfed spawned fish were also found in their intestines (Fig. 4 and 5). Degeneration of the epithelial cells was visible in only two samples, both of which came from unfed fish. A variable increase in the number of goblet cells from that seen in the "sea-green" sample was observed, but no distinction between fed and unfed spawned fish was detected.

LIVER. The livers of all the spent fish that fed actively during and following spawning, with two exceptions, exhibited atrophy but no visible degeneration (Fig. 6). In two samples, however, pycnotic nuclei and some cytoplasmic loss was observed. In the remaining fed fish (two of which ceased feeding prior to spawning) the liver displayed varying degrees of degeneration. The nuclei in some instances contained little chromatin material, while in others they were pychotic or absent. These nuclear changes were accompanied by slight, and occasionally extensive, loss of cytoplasm. The livers of the unfed spent fish resembled those of the two fish which had ceased feeding prior to spawning, except that the state of degeneration was generally more advanced with focal dissolution a common finding.

DISCUSSION

The present studies were undertaken as a preliminary to more detailed investigation of post-spawning death in Pacific salmon. Conditions were obtained under which sockeye salmon matured in captivity in outwardly good condition. However, from the data of Halver's group on the nutritional requirements of salmonoid fishes (Halver, 1963), it appears very probable that the diet of the fed sockeye salmon was deficient in protein and vitamins. The amounts of zooplankton eaten daily contained less of these nutrients (unpublished analyses) than fish of their size would require, assuming that results on juvenile fish can be extrapolated to sexually ripening salmon. A deficient diet is a possible explanation for the similar degree of muscle degeneration in fed and unfed fish. The deterioration of the lateral muscle is of interest in view of the findings of Robertson and Wexler (1960). These investigators did not observe this change in their study of adult Pacific salmon. In an investigation with immature rainbow trout (*Salmo gairdneri*), however, Robertson *et al.* (1963)

found essentially normal-appearing lateral muscle following 56 days of starvation, but extensive degeneration after 94 days. Hence, the deterioration of this muscle noted in this study may have resulted from the somewhat delayed and prolonged spawning period (147th to the 267th day of captivity (see above)). However, at this stage, the important observation is that the fish will eat zooplankton in fresh water soon after capture and that they will continue to eat up to, during, and after spawning. Furthermore, the histological studies show that the alimentary tracts of feeding fish were maintained in much better condition than were those of nonfeeding fish. In future experiments, of course, a diet of zooplankton, which is important in inducing the fish to start feeding, will be supplemented with proteins and vitamins.

In view of the limitations noted above, it would be unwise to draw any firm conclusions at this time as to the effect of feeding on the survival of spent sockeye. The general behavior and the somewhat extended survival of some of the feeding fish (no unfed fish survived more than 2 weeks, whereas 15 to 17 fed fish survived for periods of 2–12 weeks) would suggest, however, that feeding has had at least some beneficial effect.

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Studies on the Quality of Newfoundland Cod. 11. Thaw-Drip in Polyphosphate-Treated and Untreated Fillets[1]

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ABSTRACT

Two laboratory methods used to determine thaw-drip were compared: a still-air defrost method in which samples are thawed at 3–5°C so as to permit no contact between the drip and the fish, and a water-immersion method in which samples are thawed in a plastic bag at 20–21°C. Using pre-rigor frozen, paired fillets of Newfoundland trap-caught cod (*Gadus morhua*), it was shown that it is feasible to use the air-defrost method to compare the amount of thaw-drip from polyphosphate-treated and untreated fillets.

The still-air defrost method using weight determinations gave a truer absolute value of thaw-drip. Both methods showed that polyphosphate treatment reduced thaw-drip but the water-immersion method showed a greater percentage reduction in thaw-drip as a result of treatment.

The work clarifies a point concerning the variability of uptake of polyphosphate and the difficulty in getting reproducible results even using paired fillets. This points up a serious limitation to its commercial application and throws serious doubt on the use of large numbers of fish as a statistical approach to the assessment of the effect of polyphosphate.

INTRODUCTION

Empey (1933) showed that for meat, thaw-drip has approximately the same composition as the expressed juice of the unfrozen muscle in which the protein is almost entirely myogen (normally contained in the muscle cell). Protein denaturation or insolubility may well accompany the production of drip but it is not the cause of its development (Reay, 1934). Low pH (6.2–6.4) in post-rigor muscle of fish is associated with the development of drip or "weep" from the thawed material (Empey and Howard, 1951; Bendall and Marsh, 1951) and also with denaturation of fish muscle juice (Finn, 1934). The main impetus for continuing studies of drip stems from weight and nutritional losses, handling

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and processing problems, and the objectionable appearance of fish that drip appreciably. Although the factors known to influence drip have been studied and attempts have been made to reduce the amount of drip, much work has been of less than maximum value because methods for measurement have not been standardized (Empey and Howard, 1951; Miyauchi, 1963). Callow (1951) emphasized that there is no method of measuring drip which is free from criticism and suggested that the best that could be done is to devise a readily reproducible method for comparative measurements and to specify very exactly the procedure adopted. In the present communication the authors report on the usefulness of two currently-used methods as a standard for the measurement of thaw-drip obtained from untreated and from sodium tripolyphosphate-treated cod (*Gadus morhua*) fillets frozen pre-rigor. The availability of these results will make it possible to assess the results of work (MacCallum *et al.*, 1964a) published since Miyauchi (1963) prepared a review of the factors affecting drip. The correct interpretation of these and other current results may have an important bearing on the use in the Canadian Atlantic fishery of sodium tripolyphosphate as a pre-freezing treatment for fillets. Moreover, the knowledge of the reliability of results to be expected using the two techniques should make it possible in the future to relate intelligently the drip characteristics of several Newfoundland fish populations with data available from other sources.

The two laboratory methods for measuring thaw-drip which are reported on are (i) the Hartshorne and Puncochar (1951) method modified by Dyer *et al.* (1962) and here defined as the still-air defrost method, and (ii) the water-immersion method used by U. S. Fish and Wildlife Inspectors (Anon., 1961). In this study the amounts of drip collected from *paired*, pre-rigor frozen cod fillets were measured.

EXPERIMENTAL

Thawing Techniques

In the still-air defrost method, a weighed 50–100-g sample of frozen fish is placed on a sieve supported in a funnel which drains into a graduated cylinder. The entire apparatus is tightly enclosed in a large plastic bag which prevents evaporation. The sample is thawed at 3–5°C over a 48-hr period. At the end of this period the volume of the drip is read and the percentage thaw-drip calculated. As a check, the thawed fish may be weighed and the percentage thaw-drip calculated by weight. At no time during thawing does the fish come in contact with the drip; therefore, no reabsorption can occur. Thaw-drip reported by this method may therefore be defined as the amount of fluid observed to separate free of the sample when the latter is allowed to thaw at 3–5°C for 48 hr, the only external force employed being that of gravity. At any given time the volume of drip can be recorded and the percentage calculated.

The water-immersion method permits the fish to remain in contact with the drip so that the sample may regain weight. Thaw-drip or percentage by weight of "free drip" determined by this method is defined as "the fluid which

is not reabsorbed by the fish tissue when the frozen fish thaws, and which separates freely without the aid of any external force other than gravity." In this procedure a stainless steel bar and large plastic bag are first weighed. A sample of frozen fish (50–100 g for comparison) is placed in the bag which is again weighed. The bag is sealed and placed in a circulating water bath at 20 ± 1.1°C. When the fish is thawed approximately ½ hr later the bag is dried on the outside, then opened and the whole contents allowed to drain on a weighed sieve for 2 min. The bag and bar are then removed. The sieve and the fish retained by it are weighed and the percentage thaw-drip calculated.

Preparing and Thawing Samples

Five female and one male cod (56–61 cm long) were taken at a trap near St. John's at the end of June 1963 (surface water temperature 5.5°C). Each fish was stunned, bled, and the pH in the flesh near the dorsal fin measured using a glass electrode. Each fish was gutted, the belly cavity washed in clean sea water, and the fish buried in flaked freshwater ice. The pH of each of these pre-rigor fish was read at the plant about 5 hr later (Table I); each fish was filleted and skinned. One fillet of each pair was dipped in a 10.7% solution of sodium tripolyphosphate used in the weight ratio of 1 part solution to 14 parts fillet. The other fillet of each fish was not treated. Half the tripolyphosphate solution required for three fillets was poured into the bottom of an enamelled tray, the three fillets laid in the solution, and the remaining portion of the liquid poured as evenly as possible over the fillets. The fillets were individually lifted out, turned over and reintroduced into the solution by hand for a period of 2 min, at which time practically all of the solution was absorbed by the fish. The procedure was repeated for three more fillets. The fillets were cut crosswise for packing and all pieces with the exception of a small tail portion in sample No. 5 (Table I) were packed in 12-oz waxed paper cartons. Pieces from one fillet only were used in any one carton. The cartons were placed in a commercial horizontal plate freezer at Job's Southside Plant and frozen to −23°C (−10°F) in about 2¼ hr.

After 1 week frozen storage at −23°C duplicate samples were thawed and the amount of drip determined according to the still-air defrost method. At various intervals throughout a 72-hr period the volume of drip was taken, the percentage thaw-drip calculated and plotted against time (Fig. 1 and 2). The drip was weighed after the 48-hr thawing interval (Table I).

Three more trap fish of the same lot were prepared as pre-rigor frozen paired treated and untreated fillets. One week following freezing, duplicate determinations for drip were made on treated and untreated samples using the water-immersion method (Table I).

Undipped paired fillets of three more pre-rigor trap cod of the same lot were handled similarly to the above and used to obtain data on drip using the still-air and the water-immersion methods (Table II). The values for thaw-drip determined after 2 weeks frozen storage are recorded in Table II.

TABLE I. Thaw-drip from sodium tripolyphosphate-treated and untreated fillets of pre-rigor frozen trap-caught cod.

pH in gutted, iced fish immediately prior to filleting and freezing	Fillet[a]	Percentage thaw-drip			
		Still-air thawing method (weight determinations after thawing for 48 hr)		Water-immersion method (weight determinations after thawing for ½ hr)	
		Determination		Determination	
		First	*Second*	*First*	*Second*
6.5	1	12.8	14.9		
	2	9.7	10.1		
6.8	3	6.5	6.5		
	4	7.6	5.6		
6.4	5	4.8	6.6		
	6	5.9	4.6		
6.5	7	10.0	11.7		
	8	7.9	3.1		
6.5	9	9.9	8.3		
	10	4.6	6.8		
6.5	11	9.4	8.4		
	12	8.7	6.3		
6.6	13			4.6	5.1
	14			1.4	2.5
6.7	15			6.5	6.5
	16			2.9	2.9
6.5	17			4.3	4.4
	18			2.6	6.0

[a]Odd-numbered fillets not treated; even-numbered fillets treated with sodium tripolyphosphate before freezing.

RESULTS AND DISCUSSION

The thaw-drip curves for untreated cod fillets thawed by the still-air defrost method (Fig. 1) verified previous observations (unpublished results by W. J. Dyer and by the present authors). The percentage thaw-drip remains almost constant after 48 hr but before this time the curves change continually. An important point demonstrated (Fig. 2) was that the thaw-drip curves for

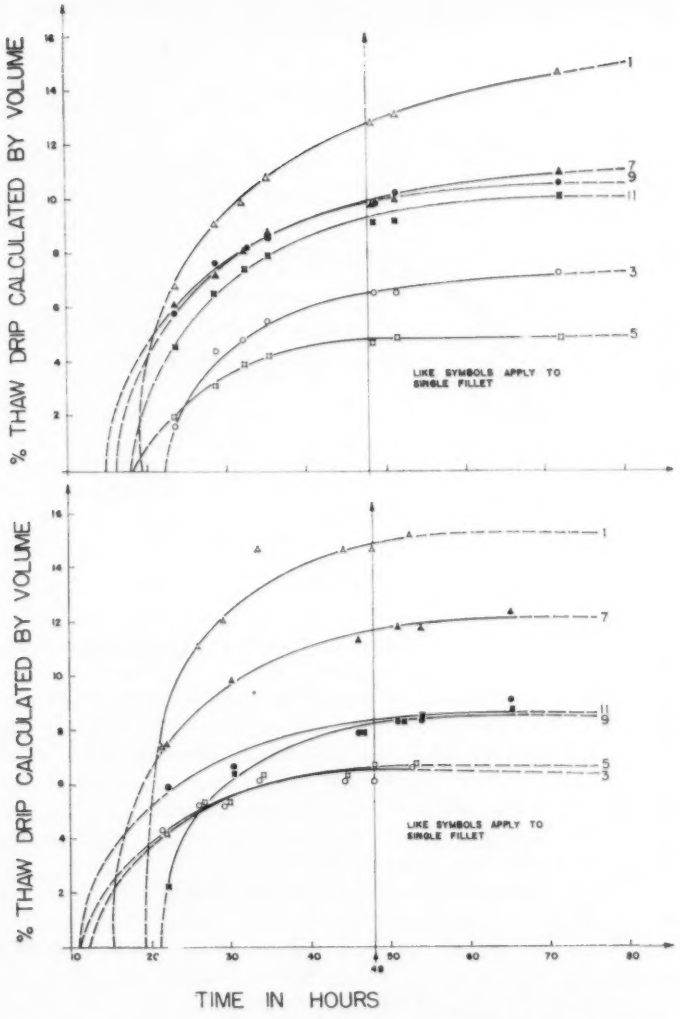
% THAW DRIP CALCULATED BY VOLUME
LIKE SYMBOLS APPLY TO SINGLE FILLET
% THAW DRIP CALCULATED BY VOLUME
LIKE SYMBOLS APPLY TO SINGLE FILLET
TIME IN HOURS

FIG. 1. Percentage thaw-drip (volumetric method) using the still-air thawing method, from six untreated fillets of pre-rigor frozen trap-caught cod. *Top*: first determination; *Bottom*: second determination. Like symbols apply to the same fillet. Table I lists the pH of the fish used to produce each fillet designated by the numbers on the curves.

the treated fillets assume the same form as those for the paired untreated samples. Therefore it is feasible to use the volume of the fluid collected after a 48-hr period as a measure of the thaw-drip from both sodium tripolyphosphate-treated and untreated, pre-rigor frozen, trap-caught cod fillets.

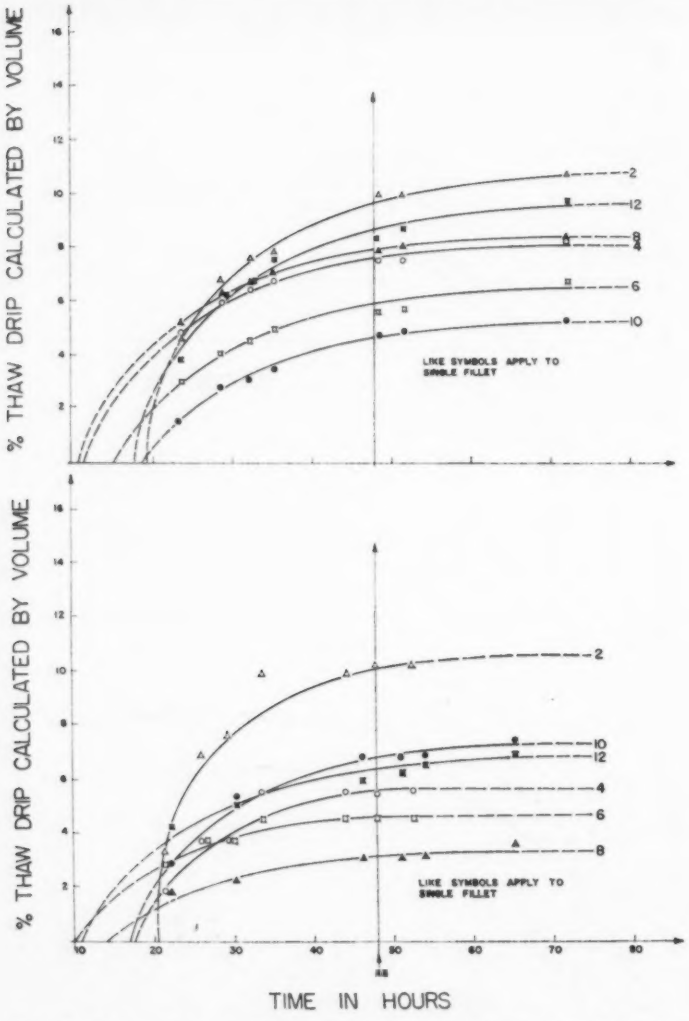

FIG. 2. Percentage thaw-drip (volumetric method), using the still-air thawing method, from six sodium tripolyphosphate-treated fillets paired with those reported in Fig. 1. *Top*: first determination; *Bottom*: second determination. Like symbols apply to the same fillet. Table I lists the pH of the fish used to produce each fillet designated by the numbers on the curves.

Among the several factors (Empey and Howard, 1951) which influence the amount of thaw-drip we shall consider the shape and weight of samples, pressure applied, the portion of fillet from whence the sample originated, and the water-holding capacity of the muscle. In this investigation, temperature and

TABLE II. Percentage thaw-drip from untreated trap-caught cod, as determined by the still-air and water-immersion methods.

pH in gutted, iced fish immediately prior to filleting and freezing	Fillet	Percentage thaw-drip (still-air thawing method)		Matching fillet	Percentage thaw-drip (water-immersion method)
		By volume	By weight		By weight
6.8	19	6.3	7.7	19A	4.2
		(8.0)[a]	(8.2)		8.2(5.7)
		9.6	8.7		4.8
6.7	21	8.1	8.0	21A	7.9
		(8.9)	(8.7)		9.2(9.0)
		9.6	9.4		10.0
6.8	23	4.9	5.8	23A	6.6
		(5.5)	(6.0)		5.8(5.8)
		6.1	6.1		5.0
Averages:		7.4	7.6		6.8
		7.5			

[a]Average values for individual fillets in parentheses.

pressure were constant for all samples and the shape and weight of the samples were kept as similar as possible. Weights ranged from 51.9–64.8 g (averaging 56.4 g), while all shapes were parallelpipeds of approximately similar dimensions. The piece of fillet used was in all cases the "thick" part 4–6 myotomes from the nape. The major variable was water-holding capacity of the protein which could be influenced by treatment with sodium tripolyphosphate (MacCallum *et al.*, 1964a, b). Despite some deviations indicated by the data (Fig. 1 and 2), the net effect of the sodium tripolyphosphate treatment was to lessen drip. A comparison was made of values obtained at 48 hr (Table I). With one exception, treated fillets showed less percentage drip and on the average, lost 23% less drip than untreated samples. The percentage difference between duplicate determinations on each sample was high, the average being 34.2% for treated and untreated samples combined. The average difference between duplicate determinations on dipped samples was 53%, on undipped 17%, or one-third as great. Thus considerable difficulty may be experienced in obtaining uniform contact of the sodium tripolyphosphate solution with all surfaces of each dipped fillet. *This points up a serious limitation to the commercial application of the method and throws serious doubt on the use of large numbers of fish as a statistical approach to the assessment of the effect of polyphosphate.*

The fact that duplicate samples from cartons 15 and 16 (Table I) developed the same thaw-drip, respectively, attests to the significance of the observation

that the effect of the polyphosphate treatment is more apparent when the water-immersion technique is used. The average drip from treated samples thawed in still air (Table I) is 6.8%; from untreated 9.1%. Corresponding average values for samples thawed in water are only about half the above, 3.1 and 5.2% (Table I).

Table II gives values of thaw-drip from paired untreated fillets thawed in air and in water, respectively. With both thawing methods the variation within the fillet was reasonably small for samples 21, 21A, and 23, 23A. With sample 19 the variation was large: 52% difference between duplicates thawed in air (using the volume method) but only 13% difference when the samples were weighed. The variation in sample 19A was still larger, 95%. The difference in results arising from the use of the two thawing methods on paired untreated fillets was not great. In two out of three instances the percentage thaw-drip was virtually the same: 3 and 6% difference only. In the other instance, samples 19 and 19A, the difference was 44% which was less than the variation experienced within the sample itself (Table II). Thus it would appear that the drip exuded from untreated fillets at the higher temperature (water defrost for ½ hr at 20°C) was not reabsorbed by the fillet. This however was not the case for the treated and untreated samples referred to in the preceding paragraph. Hence no firm conclusion can be reached to the effect that less thaw-drip will be freed when untreated, pre-rigor frozen fillets are thawed in a bag in water, rather than in still air, but there are indications that this may be so.

A study of all the data in Table II shows that considerably greater precision was achieved when the drip from still-air thawed samples was weighed rather than measured volumetrically. The still-air defrost method is preferred where time is not an important factor but the water-immersion system might be more practical for use in some quality control procedures where the time factor is important.

In the future, this study on pre-rigor frozen trap cod should be correlated with work on in-rigor and post-rigor frozen trap fish in which the pH at freezing can be expected to be in the range (MacCallum *et al.*, unpublished data) which engenders maximum development of thaw-drip.

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Phosphatase Activity in Some Species of Marine Phytoplankters[1]

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ABSTRACT

Evidence has been obtained for acid phosphatase activity (on *p*-nitrophenyl phosphate as substrate) at pH 4.8 in cell-free extracts of *Phaeodactylum tricornutum*, *Skeletonema costatum*, *Cyclotella nana*, *Monochrysis lutheri*, *Isochrysis galbana*, and *Dunaliella tertiolecta* grown photoautotrophically in pure culture. No alkaline phosphatase activity at pH 10.5 was observed.

INTRODUCTION

In a recent review on algal enzyme systems, Jacobi (1962) has drawn attention to the need for direct examination of enzymes in a variety of unicellular algae in order to substantiate the presumed existence of the metabolism patterns established in higher plants and the few algal species routinely studied. The unique taxonomic position of unicellular algae at the crossroads of evolution leading from bacteria (or their ancestors) to plants and animals suggests that their enzymatic make-up may be transitional in resembling that of any of these major groups of organisms with the possibility of unique differences developing in the more specialized algal forms (e.g. diatoms).

The availability of some marine phytoplankters in pure culture has offered the opportunity to make a preliminary examination of certain arbitrarily chosen enzyme activities in organisms belonging to several classes of algae. In the present paper, the results of such an examination of phosphatase activity in three species of diatoms, two species of Chrysophyceae, and one species of Chlorophyceae are presented. Crude cell-free extracts of the algae were used for the examination. Since most enzyme assays depend on spectrophotometric measurement, it was preferred to prepare the extracts relatively free from algal pigment color by cold acetone treatment of dried algal cells followed by buffer extraction of the resultant acetone powders. Holzer and Holzer (1952) have used this method to prepare active enzyme extracts from *Chlorella* cells.

MATERIALS AND METHODS

Algal Species Studied

Bacillariophyceae. *Phaeodactylum tricornutum* Bohlin (Lewin strain from Woods Hole, Mass.); *Skeletonema costatum* (Grev.) Cleve (clone "Skel" from Woods Hole, Mass.); *Cyclotella nana* Hustedt (estuarine clone 3H from Woods Hole, Mass.).

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CHRYSOPHYCEAE. *Monochrysis lutheri* Droop (Millport strain obtained via Haskins Laboratory, New York); *Isochrysis galbana* Parke (clone "Iso" from Woods Hole, Mass.).

CHLOROPHYCEAE. *Dunaliella tertiolecta* Butcher (clone "Dun" from Woods Hole, Mass.).

All species were obtained in pure culture from the sources indicated. Stock cultures were maintained by 2- to 3-weekly transfer to a medium (PPCMM-2) made up from open ocean seawater diluted to salinity of ca. 26‰ and buffered at pH 7.6–7.8 (TRIS 8.3 mM/litre medium) after the following nutrient additions (μM/litre medium): nitrate 500; orthophosphate 50; metasilicate 300; vitamins (B_{12} 13.4×10^{-4}; thiamine.HCl 14.8×10^{-1}; biotin 4.1×10^{-3}); and chelated trace metal ions (Fe^{3+} 10; Mn^{2+} 5; Zn^{2+} 2; Mo^{2+} 1; Cu^{2+} 0.1; Co^{2+} 0.05; EDTA 21.8).

CULTURE CONDITIONS

All organisms were mass-cultured aseptically in conical flasks under continuous illumination, with the temperature controlled at 20–25°C.

The diatoms and *Dunaliella* were cultured in 2–3 litres of medium with continuous aeration (air enriched with 5% CO_2), mechanical agitation (magnetic stirring bar), and lateral illumination (ca. 1600 ft-c) from incandescent lamps. The Chrysophyceae were cultured in 10 litres of medium equally distributed in ten flasks, shaken continually (rotatory shaking machine) without aeration, and illuminated from above by a battery of "cool white" fluorescent tubes.

The composition of the mass culture medium differed from that of PPCMM-2 (see above) in containing the following enhanced nutrient enrichments and in being buffered at pH 7.9–8.1. The silicate enrichment was increased to 1000 μM/litre for diatoms and to 500 μM/litre for the other species. All other nutrient enrichments were increased to twice those of PPCMM-2. In order to avoid any possible toxic effects, only half the phosphate was added initially and the remainder added after appreciable growth had occurred.

Cultures were initiated with inocula of ca. 1×10^6 to 3×10^6 cells per litre of medium. Growth was followed by periodic cell counts (hemacytometer). Sterility checks were made with 21 days incubation (room temperature) in the STP medium of Provasoli *et al.* (1957); negative tests were obtained from all the cultures and were considered to indicate the absence of bacterial contamination.

CELL COLLECTION

Algal cells were generally collected by continuous centrifugation (Servall RC-2 model fitted with a "Szent-Gyorgi & Blum" 8-tube continuous flow system) at ca. 25,000 *g* and 7–8°C. The collected cellular mass was successively washed with cold synthetic seawater of salinity 30, 20, and 10‰, by stirring to resuspend cells, followed by high-speed centrifugation at 0°C and decanting off the clear supernatant fluid. The cell residue from the final wash was dried for

about 1–2 days with P_2O_5 in an evacuated desiccator (ca. 100 μ Hg vacuum) at 0°C. Yields of the resulting dry dark-green cellular powders are shown in Table I.

Acetone Powders

Dry cellular (200–400 mg) powder was made into a paste with 1 ml iced water and the paste dispersed in 25 ml acetone at 0°C. After standing 20 min at 0°C, the suspension was centrifuged (ca. 22,000 g) for 15–30 min at 0°C, and the dark-green supernatant was discarded. The cellular residue was twice re-treated with acetone as before and then dried overnight in an evacuated desiccator with P_2O_5 at 0°C. The resulting acetone powders (yield 60–80% of dry cell weight) were stored in an evacuated container with desiccant at −30°C.

Enzyme Extracts

Sodium orthophosphate buffer (0.019M, pH 7.5) was generally used for extracting enzymes from acetone powders. Two other buffers used in this connection were an acid buffer (0.02M sodium citrate, pH 5.5) and an alkaline buffer (0.1M LiCl + 0.05M $KHCO_3$ + 0.001M EDTA, pH 8.5). One hundred milligrams acetone powder was gently shaken with 2 ml buffer at 0°C for 2 hr, the suspension was centrifuged (ca. 22,000 g) at 0°C for 1 hr, the clear supernatant was collected and its volume made up to 2 ml with cold buffer. This supernatant was stored at 0–4°C for periods up to a week and was used for enzyme assays as required. The phosphatase assays were usually carried out 1–2 hr after preparation of the enzyme extracts.

Phosphatase Assay

Phosphatase activity was assayed by procedures based on the method of Bessey *et al.* (1946), using disodium *p*-nitrophenyl phosphate (Sigma 104) as

Table I. Counts and yields of cells collected from mass cultures.

	Cell count ($\times 10^6$/ml)	Cell yield (dry wt (*mg*) per litre culture)
Phaeodactylum tr.	11.9	338
Skeletonema co.	12.0	217
Cyclotella na.	21.5	413
Isochrysis ga.	3.2	41
Monochrysis lu.	2.9	66
Dunaliella te.	3.5	413

substrate, 0.1M sodium glycinate (+ 0.001M $MgCl_2$) buffer (pH 10.5) for alkaline phosphatase, 0.09M sodium citrate buffer (pH 4.8) for acid phosphatase, and *p*-nitrophenol as standard for preparing calibration curves.

ACID PHOSPHATASE. Algal extract, 0.1 ml, was incubated with 0.25 ml 0.014M substrate solution and 0.25 ml buffer at 37–38°C for periods up to 30 min. Enzyme activity was stopped by addition of 2.5 ml 0.1N NaOH and the optical density was read at 410 mμ (Beckman DU) against a reagent blank. Correction for color of algal extracts was made by taking suitable blanks without substrate.

ALKALINE PHOSPHATASE. Incubation was effected as before with a mixture of 0.1 ml algal extract, 0.5 ml substrate solution, and 0.5 ml buffer, and was stopped by addition of 10 ml 0.02N NaOH. The optical density was read as before and was corrected for extract color by adding 0.1 ml conc. HCl and reading again.

The unit of enzyme activity was taken as that required to liberate 1 μM *p*-nitrophenol per hour under the specified conditions.

The protein concentrations in the enzyme extracts were measured following the method of Lowry *et al.* (1951), using crystalline bovine serum albumin (Sigma) and crystalline rabbit muscle aldolase (Sigma) as standards.

RESULTS AND DISCUSSION

The physiological state of some of the cultures at the time of cell collection is indicated by the growth curves (Fig. 1). Culture conditions were chosen so as to obtain maximum cell yields with excess of nutrients and with light only limiting growth from ultimate overcrowding. As far as possible, cultures were harvested towards the end of (or shortly after) the logarithmic phase of growth. Cell yields were markedly lower with cultures shaken without aeration (Table I).

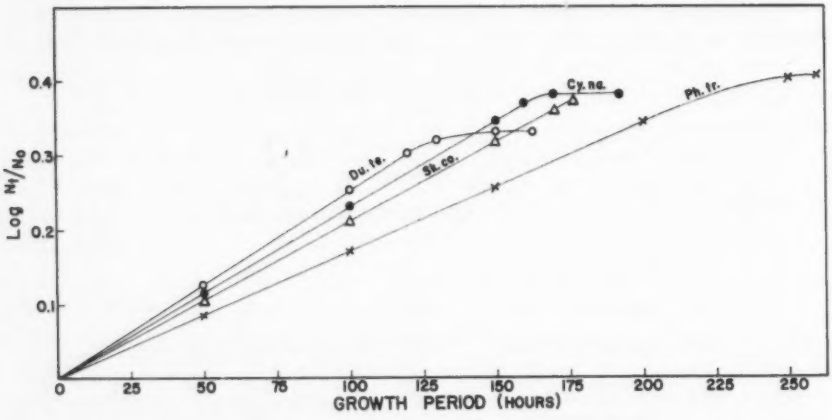

FIG. 1. Mass culture growth curves based on cell counts. (*Nt* and *No* denote number of cells per millilitre at time *t* and zero, respectively. Organisms are designated by their name initials. Cells were harvested at terminal points of the curves.)

The enzyme assays indicated acid phosphatase activity in crude extracts from all the organisms examined. The preliminary evidence for this activity is presented in Fig. 2, which shows linear rate of nitrophenol formation from substrate hydrolysis by enzyme action. The activities calculated per milligram protein extracted are summarized in Table II. With *Dunaliella*, the activity observed in the acid buffer extract is significantly greater than in the phosphate and alkaline buffer extracts. *Phaeodactylum*, however, showed no such pH-dependent effect of buffer on extraction of enzyme activity. The remarkably high activity observed in *Skeletonema*, *Isochrysis*, and *Dunaliella* suggest an important role of nonspecific acid phosphomonoesterases in the metabolism of these organisms. Studies of the activity on natural substrates (e.g. glycerophosphate) will be required to assess such a role. Although nonspecific acid phosphatases have been found to occur in a wide variety of organisms ranging from

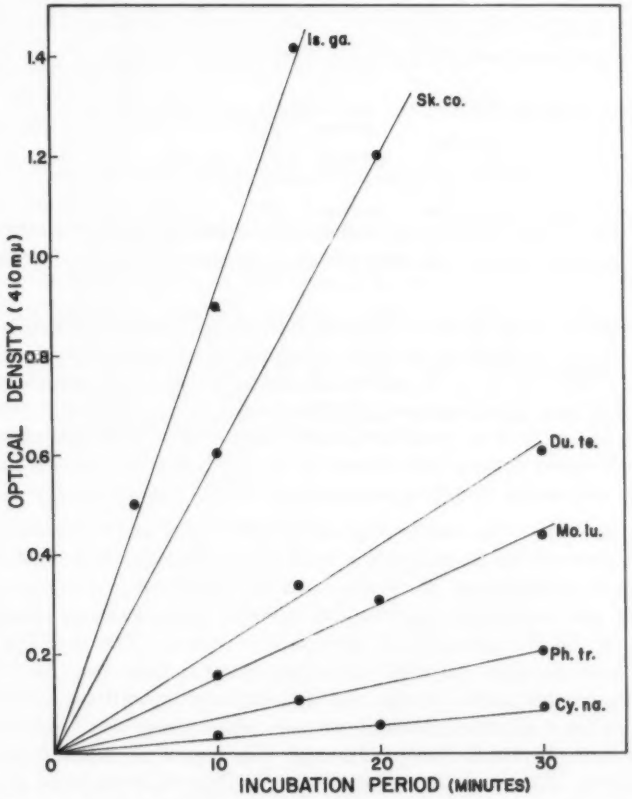

FIG. 2. Rate of substrate hydrolysis by 0.1-ml algal extracts under conditions of acid phosphatase assay. (Name initials used to designate organisms. The *Dunaliella* extract was prepared with acid buffer, all other extracts with phosphate buffer.)

TABLE II. Protein content and acid phosphatase activity of algal extracts.

		Extraction buffer	Protein (mg/ml)	Acid phosphatase activity	
				Units/ml	Units/mg protein
Phaeodactylum tr.		Phosphate	1.7	0.4	0.2
		Acid	1.2	0.8	0.7
		Alkaline	1.8	1.2	0.7
Skeletonema	*co.*	Phosphate	2.0	6.3	3.2
Cyclotella	*na.*	"	1.4	0.3	0.2
Isochrysis	*ga.*	"	4.1	10.5	2.6
Monochrysis	*lu.*	"	5.8	1.6	0.3
Dunaliella	*te.*	"	0.9	1.8	2.0
		Acid	0.8	2.5	3.3
		Alkaline	1.0	1.5	1.5

bacteria, yeasts, and moulds to higher plants and animals (cf. Boyer *et al.*, 1961a), they appear to have diverse and complex metabolic functions not clearly understood.

No alkaline phosphatase activity was detected in any of the algal extracts examined. Other methods of enzyme extraction and assay will be required to verify this observation, as it is possible that low levels of activity with pH optima at 8–9 may have escaped detection or may have become inhibited by a component of the buffers used for enzyme extraction. Alkaline phosphatases from some natural sources are known to be inhibited by certain anions (e.g. phosphate) and metal-chelating agents (e.g. EDTA) (see Boyer *et al.*, 1961b).

It appears from the results that unicellular algae of the classes examined resemble higher plants in possessing acid phosphatase(s) and relatively little or no alkaline phosphatase (cf. Naganna *et al.*, 1955). Such a comparison may be valid for the particular physiological state of the organisms obtained with active growth in the presence of abundant nutrition. The possibility is not precluded however that alkaline phosphatase formation may be induced or enhanced by growth under certain nutrient-deficient conditions. Some bacteria are known to form alkaline phosphatase only when phosphate becomes limiting for growth (e.g. Torriani, 1960), although this is not a common property of microorganisms (Kuo and Blumenthal, 1961). That diatoms may possess this property is suggested by a recently reported observation of release of alkaline phosphatase into culture medium by *Phaeodactylum* cells starved of phosphate (Kuenzler *et al.*, 1963).

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Water Exchange in Port Moody, British Columbia, and Its Effect on Waste Disposal[1,2]

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ABSTRACT

Port Moody is a comparatively shallow appendage of the Burrard Inlet–Indian Arm system on the southern coast of British Columbia. Although highly stratified, it receives little direct run-off, its oceanography being strongly influenced by the properties of contiguous waters. Wastes from various industries surrounding the harbour have altered the natural characteristics and created mildly polluted conditions. Exchange occurs mainly by turbulent diffusion and advection arising out of tidal action. From current measurements at the entrance to Port Moody, taken on a number of surveys since 1957, net currents were evaluated for periods of a tidal day. A layer of no net motion near 10 m corresponded to a dissolved oxygen minimum. Volume transport through different layers of the harbour was estimated from current data and compared with transport derived from predicted tide heights. The ratios of predicted to measured transport averaged 0.77 on both flood and ebb but ranged from 0.71 to 1.01 on the flood and from 0.55 to 1.00 on the ebb. Estimates of flushing rates, based on exchange ratios in different layers of Port Moody, show that for a survey in September 1962 slightly more than 50% of an introduced contaminant was removed from the upper 12.5-m layer, 83% from the 12.5–17.5-m layer, and 74% from the near-bottom layer, in one tidal day.

INTRODUCTION

The body of water commonly referred to as Port Moody is an appendage to the Burrard Inlet–Indian Arm system, and is essentially a deep-sea harbour (Fig. 1). It has received little interest from oceanographers in the past because it is not representative of typical inlets along the coast of British Columbia, and its natural characteristics have been considerably modified by man-made effects.

Being primarily concerned with the effects of industrial wastes on the marine waters of Port Moody and the possible consequences of this pollution in other parts of Burrard Inlet, we undertook annual oceanographic surveys, starting in 1957. These surveys were carried out usually during the late summer and early autumn when the seasonal effects of pollution can be expected to be most pronounced. The investigation was part of the monitoring program in the estuarine waters along the so-called Lower Mainland of British Columbia. A series of regular oceanographic stations was occupied throughout Burrard Inlet into Port Moody, appropriately spaced to give the spatial distribution of properties and changes that occur both longitudinally and transversely in the inlet. Because water exchange in Port Moody is an important factor with respect

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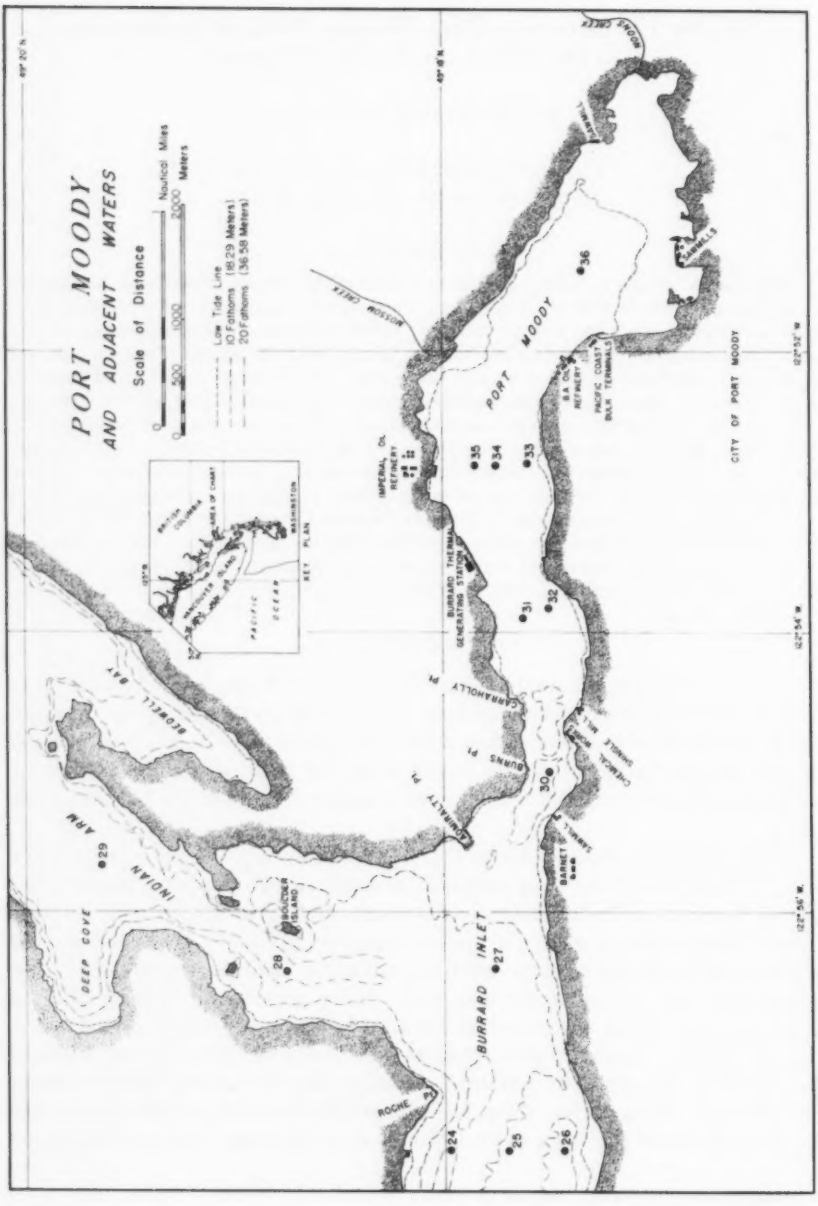

FIG. 1. Chart of Port Moody and adjacent waters, showing stations occupied in a survey, September 1962.

to future disposal of wastes, current measurements were conducted at the entrance to Port Moody, just off Burns Point (stn. 30, Fig. 1), during each survey. The present study involves an analysis of these current observations in relation to the distribution of properties and the exchange of water in Port Moody.

There is not much background information on Port Moody even though oceanographic observations have been made from time to time in Burrard Inlet and Indian Arm. As early as 1931, an investigation of seasonal oceanographic changes was made in the area (unpublished data), but coverage did not include Port Moody. Carter (1934) reviewed oceanographic characteristics of these waters in comparison with other investigated British Columbia fiords. In the sewerage program for the City of Vancouver and surrounding district, oceanographic surveys were conducted along the Fraser River estuary and in Burrard Inlet during the period 1949–51 (Pacific Oceanographic Group, 1951). Campbell (MS, 1954) carried out a study of the lateral circulation in Burrard Inlet using a theoretical approach but he did not include the area beyond the Second Narrows in Vancouver Harbour. There has been, in recent years, a number of private consultation reports on both the physical oceanography of Port Moody and the biology of invertebrates present there. A surface current study was carried out in 1957 by the Greater Vancouver Sewerage and Drainage District to determine the circulation of surface waters in the harbour, as an aid to locating new sewer outfalls from Port Moody and Coquitlam (Patrick, 1957). A study of inlets along the British Columbia mainland coast by Pickard (1961) included Indian Arm for comparative purposes. The annual cyclic changes in physical and chemical characteristics were reported by Gilmartin (1962).

GENERAL DESCRIPTION OF PORT MOODY

Port Moody is a short inlet with a comparatively small drainage area, yielding little local run-off and no inflow from large permanent streams. There are only two small creeks entering into the head of the bay and a number of drainage ditches which discharge the run-off from the local terrain. A complete assessment of the total fresh water entering into Port Moody from these sources has not been made, but this volume is relatively small compared to the run-off received by inlets having larger river tributaries. In the summertime particularly, when much of the natural run-off is reduced to zero, the evaporation may actually exceed the precipitation and run-off.

On its shores, Port Moody has several sawmills, two petroleum refineries, two chemical plants, a steel-pickling plant, and a thermal-electric generating station. All these industries discharge their various types of wastes, having different qualities, directly into the inlet. In addition to industrial wastes, there is a discharge of sanitary sewage effluent, after sedimentation of collected sewage from the communities of Port Moody and Coquitlam.

Winds in Port Moody blow generally along the axis of the inlet. Prevailing winds are from the east, although there is a fairly high percentage frequency of westerly winds during the summer. In winter, winds are easterly for about 40%

FIG. 1. Chart of Port Moody and adjacent waters, showing stations occupied in a survey, September 1962.

of the time associated with movement of air masses from the continent. The climate in general is maritime, however, and precipitation amounts to approximately 60 inches (152 cm) per year (Canada Dept. of Transport, 1963).

Similar to the remainder of Burrard Inlet, Port Moody is a comparatively shallow body of water. The inlet was classified by Carter (1934) as a *fiard*, as opposed to the typical deep British Columbia *fiord*, represented by Indian Arm. However, Port Moody is deep enough for deep-sea navigation, the greatest depths being at the entrance, where 12–13 fm (22–24 m) are recorded on the charts. The eastern half of Port Moody shoals rapidly, and a large intertidal zone marks the eastern boundary. Consequently, the mean depth of the harbour is only 5 fm (8.8 m). The extensive tidal flats mean that a great deal of water must be brought in with every tide to fill the large volume between low tide and high tide level. Table I gives some of the dimensions and parameters for Port Moody.

Bottom materials are generally mud with patches of gravel and a certain amount of organic material coming from industrial refuse and other wastes entering the harbour. In spite of the changes wrought by the industrial wastes, certain invertebrates, including the edible Pacific crab (*Cancer magister*), have survived on the bottom and are still trapped occasionally by commercial

TABLE I. Descriptive quantities for Port Moody.

Dimension or parameter	English units	Metric units
Length	3.5 nautical miles	6.5 km
Width, average	0.5 nautical miles	0.9 km
Mean depth, at low tide	29.0 ft	8.8 m
Area, Low tide	4.149×10^7 ft^2	3.855×10^6 m^2
High tide	6.112×10^7 ft^2	5.678×10^6 m^2
Volume, Low tide, V_L	1.203×10^9 ft^3	3.407×10^7 m^3
High tide, V_H	1.715×10^9 ft^3	4.856×10^7 m^3
Tidal range, for mean small tides	3.52 ft	1.07 m
for mean large tides	9.97 ft	3.04 m
Intertidal volume (tidal prism), for mean small tides, P_L	1.808×10^8 ft^3	5.120×10^6 m^3
for mean large tides, P_H	5.116×10^8 ft^3	14.486×10^6 m^3
Ratio P_L/V_L	0.1503	
Ratio P_L/V_H	0.1054	
Ratio P_H/V_L	0.4253	
Ratio P_H/V_H	0.2983	

fishermen and sports enthusiasts. Small fishes, probably juvenile herring, were seen regularly in Port Moody during the anchor station operations when lights were cast on the water.

OCEANOGRAPHIC CHARACTERISTICS

Distribution of Variables

Port Moody does not have natural effects which modify the general oceanographic character of its waters to any large extent. It derives most of its water properties from Burrard Inlet, which provides the deep-water characteristics, and from Indian Arm, where a great deal of freshwater inflow reduces the surface salinity and gives the estuarine character. One of the geographical features which enhances the stratification in the water is its semi-confinement and protection from strong winds. This reduces water exchange and wind mixing. During the summer, local heating from insolation increases surface water temperatures and thus provides an added stratifying factor to give the characteristic density structure of high stability. In winter, local precipitation and run-off can substantially reduce the surface salinity.

The distribution of the conservative properties, salinity, temperature, and density (σ_t), are shown in Fig. 2. This is essentially a late-summer distribution, observed in September 1962, when the effects of summer heating and low run-off are still present. Surface salinity is lowest, about 23‰, at station 27 in Burrard Inlet, and increases toward the head of Port Moody. The largest salinity changes with depth occur in the upper 5 m, with much slower change down to the bottom. Evidently mixing does not penetrate too deeply in this area and contributes to the shallow stratification that occurs here both in winter and summer.

Salinity of the bottom water, at 26.5‰, indicates comparatively brackish conditions even at the depth of 25 m. The source of this deep water is in Burrard Inlet, where, at the First and Second Narrows, there is intensive mixing of low-salinity surface water with the higher-salinity deep water to form a mixture that penetrates into the deep zones of Indian Arm and Port Moody.

Temperatures followed a vertical distribution at this time of year similar to that of salinity, with the main stratification in the upper 4 or 5 m. The shallow thermocline corresponded closely to the shallow halocline. The deeper water was comparatively warm, at 12°C, indicating that there had been an introduction of warm surface water mixed with colder deep water in Burrard Inlet.

From the vertical density (σ_t) distribution, combining the effects of salinity and temperature to produce the characteristic stratified density pattern (Fig. 2), it is obvious that the waters are very stable and that essentially a "seal" is formed over the deep water by a shallow, low-density surface layer. Wastes released in the deep water probably have difficulty in penetrating this "seal" to come to the surface and, of course, the surface-discharged substances cannot be mixed downward into the deep water too easily.

Measurements for extinction coefficients were made using an Askania transparency meter (Askania-Werke AG, Berlin-Friedenau, Germany), with

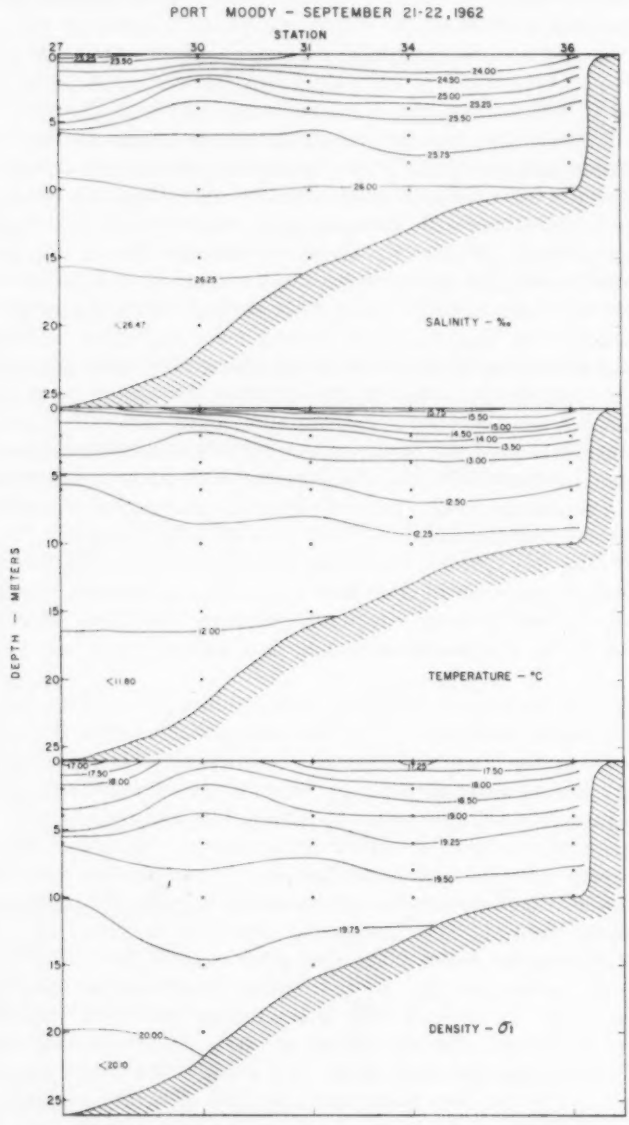

FIG. 2. Distribution of conservative properties in a vertical section through Port Moody, September 1962, along stations shown in Fig. 1.

two Jena glass filters giving light transmission maxima in the blue-green (No. BG 12 at 365–450 mμ) and in the red-green (No. RG 1 at 650–750 mμ) portions of the visible spectrum. The extinction coefficients, using only the latter filter, were computed as defined by the extinction coefficient $\xi = \log_e \frac{I_0}{I}$, where I_0 is the incident light intensity, I is the intensity of light transmitted by a thickness of 1 m of water *in situ*, and e(= 2.7183) is the base for natural logarithms. There was a large variation in the extinction coefficients vertically and horizontally from Burrard Inlet to the head of Port Moody during the survey of September 1962 (Fig. 3). The surface water was quite turbid, the Secchi disc readings being reduced from about 6 m in Burrard Inlet proper to 4 m in Port Moody. The water at about 5 m appeared to be highly transparent, judging by the near-zero extinction coefficients and presumably was of fairly recent origin from Burrard Inlet. There were two areas of deep-water turbidity which may be associated with stirring up of the bottom and/or with settling materials that tend to remain in suspension there. These deep-water cells of high turbidity are probably associated with currents near the bottom.

The dissolved oxygen distribution (Fig. 3) is typical of that observed in other surveys, with high stratification at the upper end of Port Moody. Surface waters always appear to have ample dissolved oxygen and, in fact, at certain times are supersaturated. This is no doubt due to the photosynthesis occurring in the shallow surface layer at certain times of year. Deep water entering from Burrard Inlet is relatively low in dissolved oxygen, but there was a tongue of

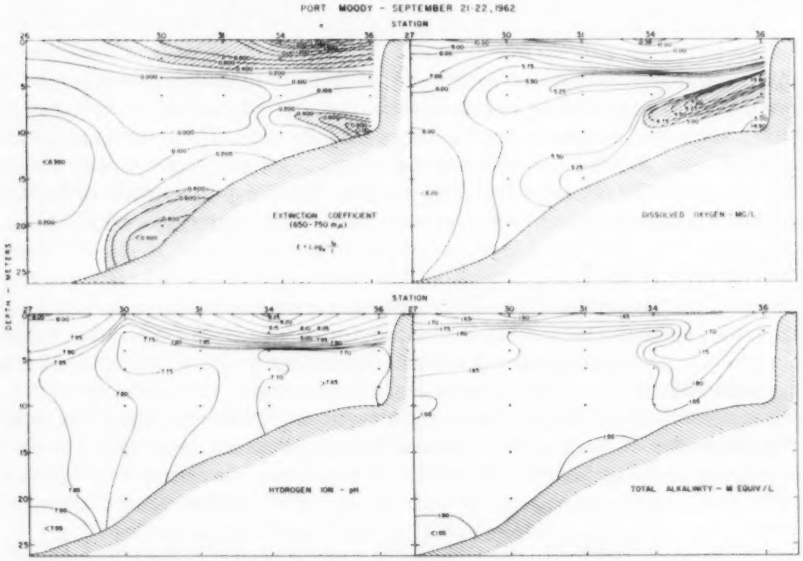

FIG. 3. Distribution of nonconservative properties in a vertical section through Port Moody, September 1962 (see Fig. 1 for station locations).

very low dissolved-oxygen water at a depth of 5–10 m in the upper end of Port Moody during the survey of September 1962. This mid-water minimum is probably related to a region of stagnation with very little net movement of water. Perhaps, at the eastern end of the harbour, organic detritus and refuse settles to a depth where it is nearly neutrally buoyant and decomposes over a period of time, robbing the oxygen from the water. Distributions of both the extinction coefficient and dissolved oxygen give useful clues to the circulation of water in a vertical profile through Port Moody.

In general, the pH distribution conforms to the dissolved oxygen pattern with relatively high values of pH near the surface and much lower values in the deep water (Fig. 3). This marked vertical stratification in pH, especially in the thin upper layer of water, is not common in most of the other inlets along the British Columbia coast. Values of pH at 8.25 are high even for saline oceanic waters. In Port Moody, high pH values are probably related entirely to the utilization of carbon dioxide from the water in plant photosynthesis. They correspond closely to the area of high dissolved oxygen, and are not believed to be in any way associated with the alteration of the water by various industrial discharges having high pH. There may be some indirect effect, however, due to excessive algae growth caused by enrichment of the water with nutrient-rich sewage or industrial wastes. The deep water in Port Moody appeared to be relatively uniform in vertical pH distribution, and any small stratification present was in the horizontal direction rather than vertical. Lowest values of pH were found in the intermediate waters at the head of Port Moody where very low dissolved-oxygen values were also found.

Total alkalinity (Fig. 3) exhibits a combination of the effects of low salinity in the surface layer and the variations of pH and dissolved oxygen with depth, that are related to the photosynthesis processes and decomposition effects. There is the normal vertical distribution in the upper layer at the entrance to Port Moody and a rather distorted pattern near the head. The latter is probably associated with the regimes of dissolved oxygen and pH at the upper end of Port Moody. The changes in the carbon dioxide–bicarbonate–carbonate equilibrium probably create the unusual distribution pattern noted here.

Year-to-Year and Seasonal Changes

One of the properties in the inlet, which is most noticeable as having been altered by the polluted conditions, is the turbidity or clarity of the water. Transparency measurements with a meter have been made on only one survey and we do not have suitable data to have comparisons from previous years. However, simple observations such as that with the Secchi disc show the rapid change in clarity of the water as one proceeds up Burrard Inlet and into Port Moody (Fig. 4). The Secchi disc readings have been converted to extinction coefficients using the formula given by Sverdrup *et al.* (1946), where the extinction coefficient is equal to $\frac{1.7}{D}$, the depth, D, being given in meters for the threshold of visibility of the white 30-cm disc.

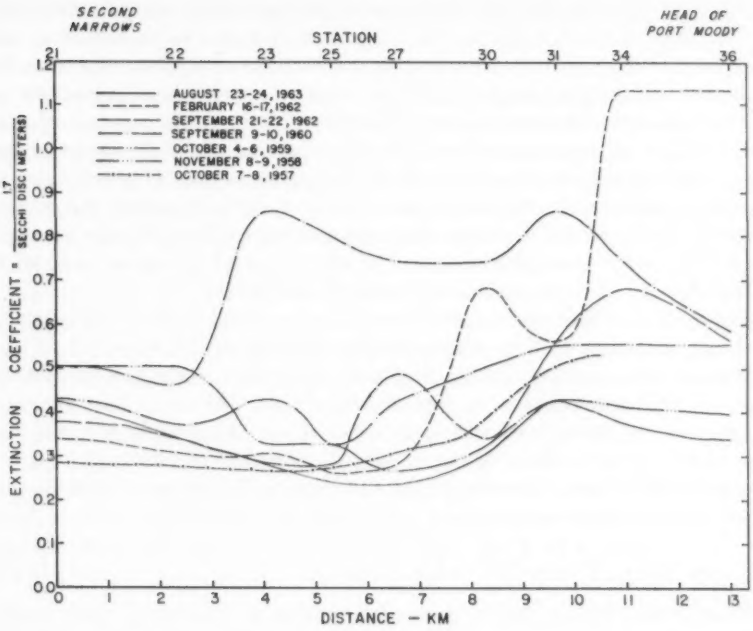
SECOND NARROWS
HEAD OF PORT MOODY
STATION
21 22 23 25 27 30 31 34 36
AUGUST 23-24, 1963
FEBRUARY 16-17, 1962
SEPTEMBER 21-22, 1962
SEPTEMBER 9-10, 1960
OCTOBER 4-6, 1959
NOVEMBER 8-9, 1958
OCTOBER 7-8, 1957
EXTINCTION COEFFICIENT = 1.7 / SECCHI DISC (METERS)
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0 1.1 1.2
0 1 2 3 4 5 6 7 8 9 10 11 12 13
DISTANCE — KM

FIG. 4. Curves showing changes in extinction coefficients, computed from Secchi disc observations, along Burrard Inlet from the Second Narrows into Port Moody, for different surveys.

There were large deviations from the normal pattern of distribution, such as in the surveys of September 1960, and February 1962; but, in general, there was an increase in extinction coefficients as one proceeded into Port Moody past station 30. Occasionally, high turbidity in the inlet is associated with run-off from the surrounding hills which brings down a considerable amount of mud from land-clearing projects and excavations in residential and industrial developments. However, the very high extinction coefficients observed during the February 1962 survey were probably due to some waste discharge, inasmuch as the survey was carried out during a relatively dry winter period. In September 1960, a large part of Burrard Inlet other than Port Moody seemed to be quite turbid, as shown in the curve for that particular survey.

The consequences of highly turbid waters are, of course, that light penetration is reduced and phytoplankton proliferation is retarded at greater depths. The phytoplankton bloom apparently occurs in very shallow layers, less than 5 m deep in Port Moody, judging by the depth at which dissolved oxygen and pH values are maximum.

The dissolved oxygen distributions in Port Moody give not only a useful index on the input of unstable organic wastes but also clues for the elucidation of circulation. Where there is rapid circulation, the water is exchanged frequently

and dissolved oxygen concentrations are maintained at a comparatively high level. In areas where circulation is sluggish and water replacement is slow, respiration by aquatic organisms and decomposition of organic materials lead to depletion of dissolved oxygen with time. Unfortunately, there are no continuous observations on water properties in Port Moody over a period of a full annual cycle to provide a picture of the seasonal changes of dissolved oxygen. Nearly a full year, however, was covered during surveys of Burrard Inlet and Indian Arm in 1931, one of the stations occupied being just outside the entrance to Port Moody. Inasmuch as water entering and leaving Port Moody must pass through this area, the information obtained is useful to show some of the seasonal changes in waters associated with Port Moody.

In Fig. 5 is shown the seasonal cycle of dissolved oxygen concentrations at a location corresponding approximately to station 27, shown in Fig. 1. There was a late summer–autumn decline from 8.0 to 6.5 mg/litre in dissolved oxygen of the intermediate waters during 1931. This seasonal minimum, however, was not too different from concentrations observed in Burrard Inlet during recent surveys. Most of the autumnal reduction in dissolved oxygen of the deeper waters is associated with the summer increase in water stability and the decrease in vertical mixing.

Tides and Tidal Currents

Tides in Port Moody are the mixed type, being the resultant of two component tides, one semi-diurnal and the other diurnal. They have a large diurnal inequality in successive low waters, which is related to the declinational effect

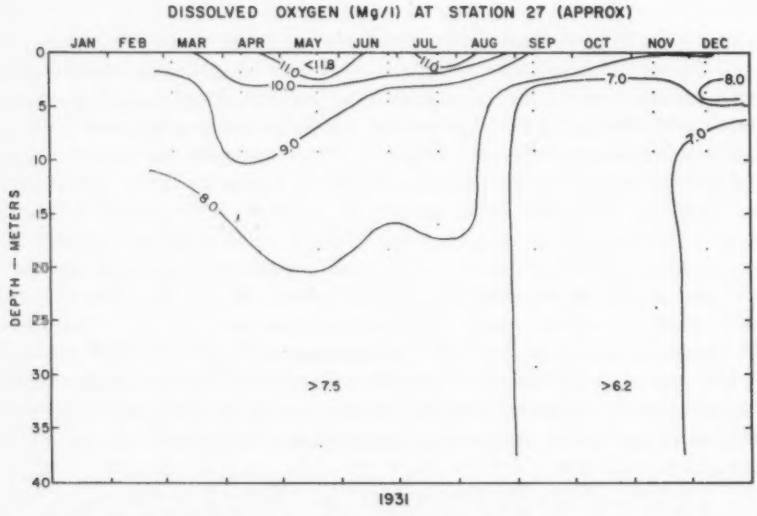

Fig. 5. Seasonal cycle of dissolved oxygen concentration at a station near the entrance to Port Moody (approx. 49°17.8′N, 122°56.4′W) during 1931.

of the moon in the sequence from equatorial to tropic and back to equatorial tides occurring at fortnightly intervals. A typical tidal cycle for a 25-hr period is shown in the tide curve (Fig. 7).

There is a lag of 15 min between the higher high water at the tide gauge in Vancouver Harbour and higher high water in Port Moody. The distance between these two points is approximately 10.5 nautical miles (20 km), which suggests that the tide advances partly as a progressive wave. However, judging by the current curves in relation to the tidal cycle, where the maximum current occurs at the mid-point between low and high waters, the tidal undulation should be nearly a standing wave. The delay in tidal rise and fall from Vancouver to Port Moody, compared with an almost instantaneous tidal change along the full length of the deep inlets, must be related to the comparative shallowness of Burrard Inlet. A considerable amount of impedance to the advance and retreat of the tidal undulation probably occurs at the First and Second Narrows, with somewhat less at the entrance to Port Moody. Evidently the tidal undulation acts partly as a forced wave (Doodson and Warburg, 1941), as it passes into Burrard Inlet, because, whereas the delay in the higher high water is 15 min between Vancouver Harbour and Port Moody, it is 25 min between lower low waters at these two points. The tidal range, however, in the two areas is identical and, therefore, the tide at Port Moody must reach an equilibrium in height with the tide in other parts of Burrard Inlet.

Surface Currents

Current surveys during many years by the Canadian Hydrographic Service on the general surface circulation in Burrard Inlet, which includes also the entrance to Port Moody, have been published in a series of Tidal Currents Charts for Vancouver Harbour (Canada Dept. Mines and Technical Surveys, 1962).

In general the current is reversing in character, moving eastward on the flood and westward on the ebb. At the entrance to Port Moody it reaches a maximum velocity of 0.7 kt (36 cm/sec) on both the maximum flood and the maximum ebb at the Second Narrows. The current weakens to about 0.2 kt (10 cm/sec) on the last of the flood at Second Narrows and undergoes a reversal to ebb just before high water. It slackens off to virtually zero on the last of the Second Narrows ebb and undergoes a reversal from westward to eastward into the Harbour.

Surface currents in Port Moody, generalized for large flood and large ebb under all wind conditions and for small flood and small ebb during calm, are reproduced in Fig. 6 from results of the work by the Greater Vancouver Sewerage and Drainage District during 1957 (Patrick, 1957). Currents on the large flood generally varied from 10 to 15 cm/sec, but some swifter flow was registered on the south side midway into Port Moody. Currents of about the same range in speed, but moving in the opposite direction, were observed on a large ebb at all points in Port Moody. The strongest current of up to 21 cm/sec

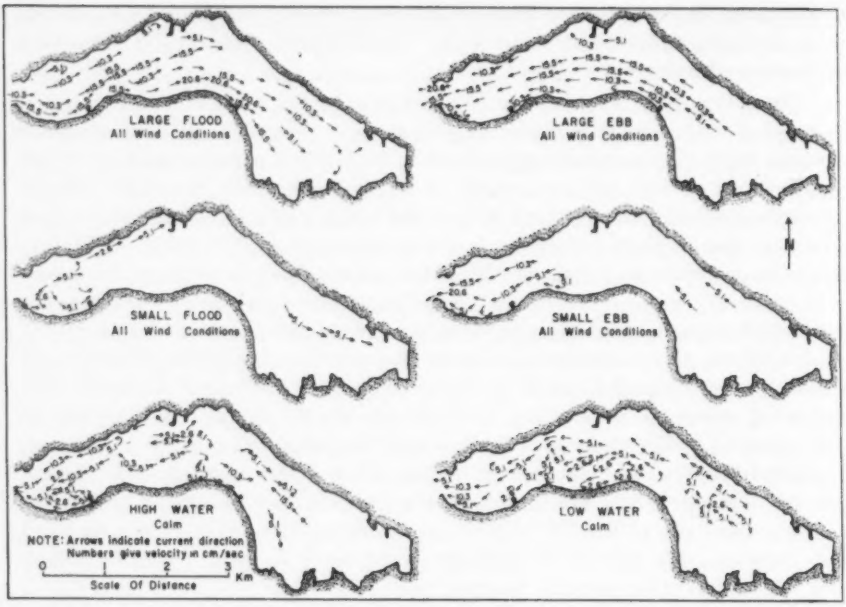

FIG. 6. Representative surface current patterns in Port Moody under different wind conditions. (From Patrick, 1957.)

was recorded at the entrance, where there was evidently a large ebb flow. Currents on the small flood were usually around 5 cm/sec and sometimes weaker with a less-defined direction of flow. Water movement was somewhat larger on a small ebb than on a small flood, being around 10–15 cm/sec with as much as 20.6 cm/sec recorded at the entrance, to Port Moody. Rather confused currents of varying speeds and directions were noted both at high water and at low water during calm periods. This is probably related to the changing tide in various parts of the harbour and adjustment to the general high- or low-water conditions.

Surface currents were observed from an anchored ship on all seven oceanographic surveys conducted in Port Moody thus far. Some difficulty was experienced in obtaining suitable current measurements using the standard technique by releasing a captive float on a line. Our last three series of continuous current observations were made with a CBI (Chesapeake Bay Institute) drag (Pritchard and Burt, 1951) which was found to give reasonably representative results, checking well with the currents measured at 5 m using the Ekman current meter.

The surface currents obtained with a CBI drag during the survey of September 1962 are shown in Fig. 7. A maximum flood current of around 30 cm/sec was attained during a moderate tidal rise. A slightly larger ebb current was registered at the surface during a similar tidal fall. The peaks in

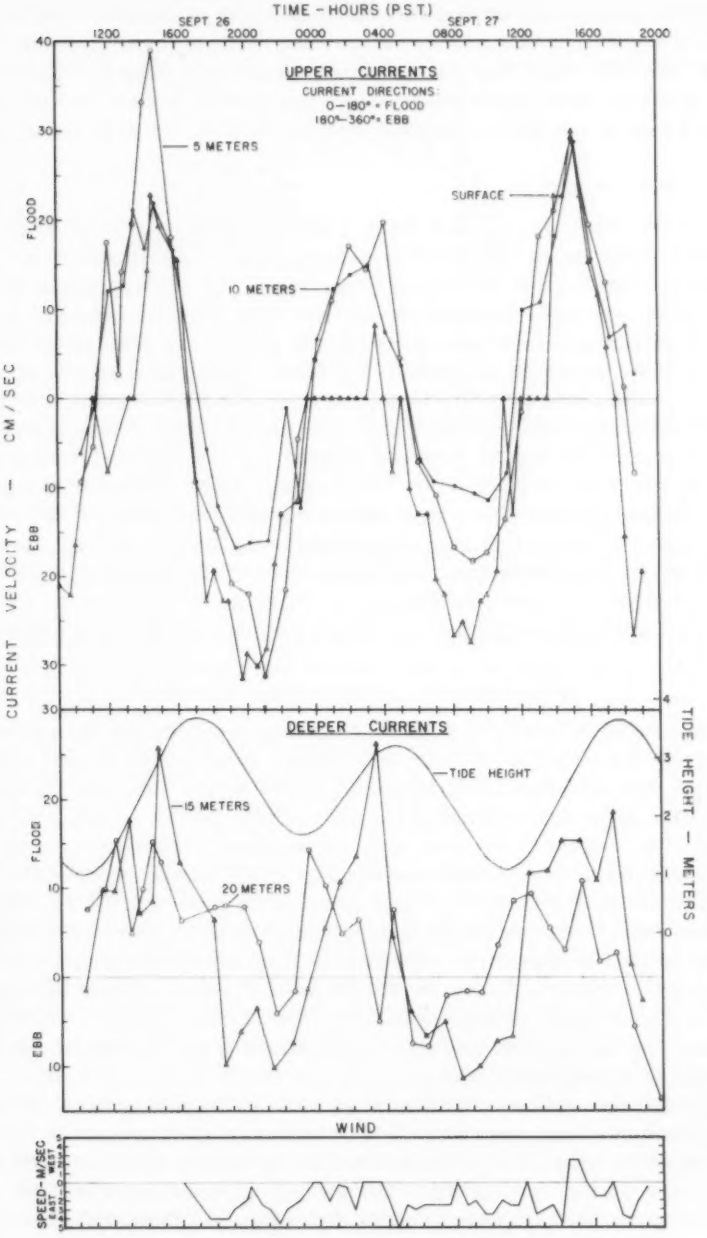

FIG. 7. A typical series of current observations taken on a 30-hr anchor station at the entrance to Port Moody (stn. 30, Fig. 1), September 1962.

the surface currents occurred during the mid-period on the tide curve between high and low waters. One of the characteristic features of the currents in Port Moody was that there was often a rapid change from flood to ebb and vice versa. However, slack water sometimes endured at the surface for long periods at the change in the tide, if the wind strongly modified the tidal movement.

Subsurface Currents

Currents at 5, 10, 15, and 20 m were measured at station 30 from an anchored vessel using an Ekman current meter. The standard technique described in the Manual of Current Observations (U. S. Department of Commerce, 1950) was used. Although the currents were small compared to those in other tidal channels, the sensitivity of the Ekman current meter allowed currents to be measured at virtually all times. Sufficient time was generally allowed for the meter propeller to spin so that at least one pellet would drop into the compartmented receptacle to provide an indication of current direction.

Currents at all depths observed showed a fairly regular cyclic pattern following the tidal curve (Fig. 7). The largest currents with maxima around 30 cm/sec were found at 5 and 10 m; the speeds and directions of these currents were not too different from those observed for the surface current. However, the deeper currents were considerably weaker. At all four depths observed there was reasonably good consistency in the phase of the currents, with the maxima occurring during the rise and fall of the tide and the slacks at high and low water.

Estimation of Inflow and Outflow

The current data were taken on all surveys primarily for the purpose of determining the exchange of water at various depths in Port Moody. Periods of at least 25 hr were covered for obtaining full cycles of a tidal day during each survey. This allowed an estimate of the net nontidal current at each level in the harbour. The current data from each survey were plotted on a time-scale (Fig. 7) and the curves were planimetered to obtain the areas under the flood and ebb portions of the curves. These areas, for a tidal day (24 hr, 50 min), were then used to determine the average flood and ebb currents during the full tidal cycle. The net current, in either the flood or ebb direction, could then be evaluated. Profiles of the net current for six of the seven current surveys are shown in Fig. 8. Unfortunately, the data from the first survey in 1957 were unsuitable for this analysis, because the station was not located at the same point used in subsequent surveys.

Although the profiles of net flow at various depths obtained from different surveys during different years show little consistency, there are certain features which are noteworthy. A net flow seaward in the surface layer occurred at all times except during two surveys. The current measurements in 1963 were taken during August at the height of the dry season and there could have been a greater inflow than outflow at the surface due to excess evaporation over precipitation and run-off. The surface current data for the 1958 survey may be

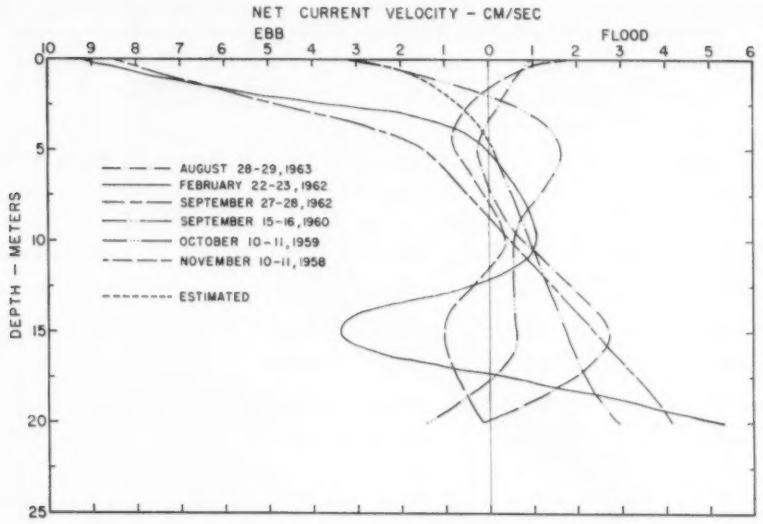

FIG. 8. Profiles of net currents obtained from measurements for a tidal day (24 hr, 50 min) during a number of anchor-station current surveys at the entrance to Port Moody.

somewhat unreliable because of the lack of a suitable technique for measurements at that time. The greatest seaward flow occurred during the survey of February 1962 when local run-off could be expected to be near its seasonal maximum. There was generally a small net seaward flow at a depth of 5 m and a small net flow inward at 10 m in all surveys. A wide variation in the directions of net flow at 10 and 15 m was apparent for the surveys represented here. There was usually a net flow into Port Moody, however, along the bottom at 20 m.

In addition to the current data, the distribution of dissolved oxygen leads to some ideas on the circulation pattern in the bay. In Fig. 9 is shown the distribution of dissolved oxygen in a section through Port Moody for February 1962. By inserting vectors for the net currents obtained from the data of current observations shown in Fig. 8 for that period, one can readily see a certain correspondence between the current and the dissolved oxygen distribution.

There appeared to be a surface of no net motion at about 5 m, which was the region of greatest vertical oxygen change, from approximately 9 to 6.5 mg/litre. The large net flow seaward in the surface layer was in line with the high dissolved oxygen concentration at this level. At 10 m there was a net flow inward, which suggests that a replenishment of intermediate water was occurring at this depth. The tongue of low dissolved oxygen evidently was in a region receiving little replacement over a considerable period of time, and the currents represented by the profiles (Fig. 8) suggest that this is, in fact, the case. A tongue of low-dissolved-oxygen water at 20 m appeared to be penetrating from Burrard

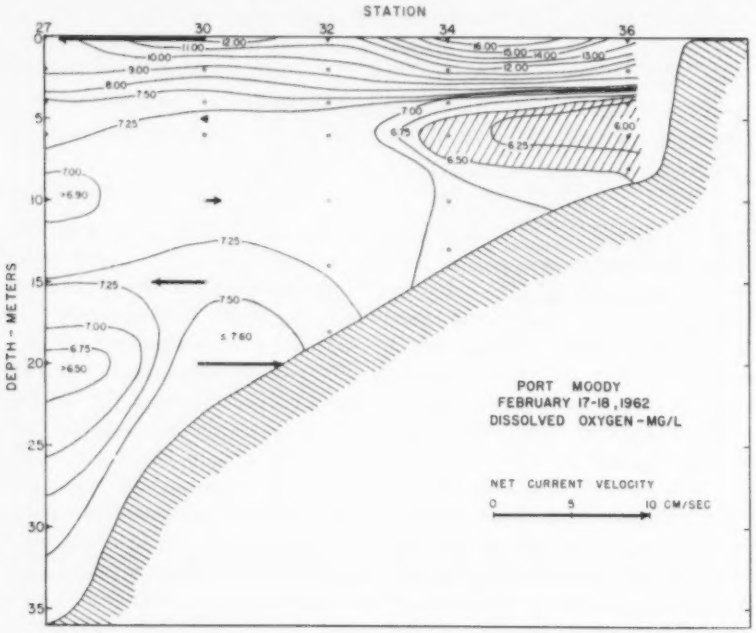

FIG. 9. Dissolved oxygen concentrations in a vertical section through Port Moody during February 1962, with vectors representing net currents during that period.

Inlet, according to the vertical distribution at station 27, and this was represented by substantial net inflow at that depth. The net outflowing water at 15 m was represented by an average dissolved oxygen content of between 7 and 7.5 mg/litre.

The current data from the various surveys have been used to compute the inflow and outflow from Port Moody. The cross-sectional area of the channel into Port Moody at station 30 was determined for various intervals of depth, 0.0–2.5 m, 2.5–7.5 m, 7.5–12.5 m, 12.5–17.5 m, 17.5 m to the bottom. The flux through these different depth intervals during a tidal day (24 hr, 50 min) was determined by using the mean tidal current velocities observed in the different layers, namely, at surface, 5, 10, 15, and 20 m. Volume transport without correction for frictional effects of the side and bottom of the channel are given in Table II. From the predicted tide heights, as given in the tide tables (Canada Dept. Mines and Technical Surveys, 1958–63) for the various periods of current measurements, and from the mean sea-level area of the inlet from Burns Point to the head of Port Moody, the volume inflows and outflows were calculated. These transports are also tabulated in Table II, in the "predicted" column. The factor of predicted to measured transports in each case gives a measure of the difference between the current velocity measured at the anchor station and the average velocity through the channel. The factor averaged 0.77 which is almost identical to the empirical value of 0.75 given in

TABLE II. Volume transport into and out of Port Moody during different surveys, as obtained from current measurements and tide-height data.

Survey period	Flood transport, T_F			Ebb transport, T_E		
	Predicted	Measured	T_F (predicted) / T_F (measured)	Predicted	Measured	T_E (predicted) / T_E (measured)
	10^7 m^3	10^7 m^3		10^7 m^3	10^7 m^3	
Nov. 10–11, 1958	2.673	3.615	0.739	2.746	3.665	0.749
Oct. 10–11, 1959	1.714	1.693	1.012	1.642	1.635	1.004
Sept. 15–16, 1960	1.366	1.895	0.721	1.366	1.785	0.765
Feb. 22–23, 1962	2.019	2.726	0.741	1.903	3.457	0.551
Sept. 27–28, 1962	2.005	2.840	0.706	1.947	3.188	0.611
Aug. 28–29, 1963	1.366	1.919	0.712	1.351	1.451	0.931
Mean:	1.857	2.448	0.772	1.826	2.530	0.769

the literature for the relationship of mid-channel currents to the average currents (U. S. Department of Commerce, 1950; Sverdrup *et al.*, 1946, p. 568). The range of factors was comparatively small, 0.71–0.74, except for the large value of 1.01 in October 1959 for the flood transports. It was much greater, 0.55–1.00, for the ebb, which may be related to variations in run-off from survey to survey.

ESTIMATES OF WATER EXCHANGE

The data given in the foregoing section can be used for an estimate of water exchange in the different layers of Port Moody. The approach used here is described by Ketchum (1951), where an exchange ratio is used to determine the flushing rate. The exchange ratio, r, has been described as the ratio of the amount of introduced material removed from a given segment of an estuary during a tidal cycle to the total amount of the material present in the segment. Fresh water has been traditionally used as a tracer in this approach. However, in this situation, where there is very little run-off, and tracers from freshwater inflow are of little use, another method must be used to define the exchange ratio. This can be done by taking the ratio of the water removed on the ebb tide, Q_e, to the total volume present in a given depth interval of the inlet, V_i. That is, $r = \frac{Q_e}{V_i}$.

If W represents the total amount of industrial effluent introduced in a given tidal cycle into the inlet, the amount removed on the first tidal cycle is rW while the amount remaining is $(1-r)$W. On the second tidal cycle, $r(1-r)$W is removed and $(1-r)^2$W remains, and on the nth tidal cycle, $r(1-r)^{n-1}$W of the effluent is removed and $(1-r)^n$W remains. As shown by Ketchum (1951), after an infinite

number of tidal cycles, the total amount of effluent, S, in the harbour under steady-state conditions can be represented by the formula $S = \frac{W}{r}$. To calculate the exchange ratio for each layer, the measured ebb transport for that layer has been corrected by the factor of predicted transport to measured transport on the ebb for the whole water column (Table II).

The exchange ratios for each depth interval on all surveys recorded here are given in Table III. Values of r greater than 1 suggest that there may be a considerable amount of vertical transport from the bottom layers. However,

TABLE III. Exchange ratios computed for the different layers of water in Port Moody during various surveys. (V_t = volume of the given depth interval; T_F = volume transport on the flood; r_f = exchange ratio for the flood; T_E = volume transport on the ebb; r_e = exchange ratio for the ebb.)

Survey period	Depth interval	V_t	T_F	r_f	T_E	r_e
	m	10^6 *m*^{3}	10^6 *m*^{3}		10^6 *m*^{3}	
Nov. 1958	0.0–2.5	10.462[a]	6.313	0.446	4.827	0.346
	2.5–7.5	13.749	10.515	0.565	11.675	0.636
	7.5–12.5	8.308	8.965	0.797	8.443	0.761
	12.5–17.5	2.737	7.044	1.902	8.302	2.272
	17.5–Bottom	0.453	3.315	5.408	3.407	5.633
Oct. 1959	0.0–2.5	10.462	3.934	0.381	6.586	0.632
	2.5–7.5	13.749	5.659	0.417	3.153	0.230
	7.5–12.5	8.308	3.151	0.384	2.353	0.284
	12.5–17.5	2.737	3.049	1.128	2.295	0.842
	17.5–Bottom	0.453	1.135	2.538	1.964	4.353
Sept. 1960	0.0–2.5	10.462	2.598	0.179	5.181	0.379
	2.5–7.5	13.749	5.350	0.281	5.183	0.288
	7.5–12.5	8.308	4.953	0.430	3.656	0.337
	12.5–17.5	2.737	4.523	1.191	2.473	0.691
	17.5–Bottom	0.453	1.524	2.426	1.355	2.288
Feb. 1962	0.0–2.5	10.462	3.607	0.255	11.413	0.601
	2.5–7.5	13.749	9.361	0.505	9.373	0.376
	7.5–12.5	8.308	7.306	0.652	5.808	0.385
	12.5–17.5	2.737	3.159	0.855	7.202	0.145
	17.5–Bottom	0.453	3.322	5.434	0.771	0.938
Sept. 1962	0.0–2.5	10.462	1.442	0.097	8.627	0.504
	2.5–7.5	13.749	9.908	0.509	12.127	0.539
	7.5–12.5	8.308	7.549	0.641	6.843	0.503
	12.5–17.5	2.737	6.584	1.698	3.730	0.832
	17.5–Bottom	0.453	2.913	4.540	0.548	0.739
Aug. 1963	0.0–2.5	10.462	4.726	0.322	3.913	0.348
	2.5–7.5	13.749	4.509	0.233	4.889	0.331
	7.5–12.5	8.308	4.470	0.383	3.399	0.381
	12.5–17.5	2.737	4.202	1.093	0.951	0.323
	17.5–Bottom	0.453	1.278	2.009	1.358	2.791

[a] The volume of the 0.0–2.5-m layer is taken from approximately mean sea level, while the deeper volumes have been calculated from the level of lowest normal tides.

small errors in current measurements and in volume determinations near the bottom may introduce large discrepancies. Flushing curves for different depth intervals during a typical period are given in Fig. 10. If it is assumed that the flushing is linear and a proportion of introduced effluent equal to the exchange ratio is removed on each tide, then the period in tidal days to remove a given volume of contaminant is given by $\frac{1}{r}$.

DISCUSSION

Perhaps one of the most remarkable characteristics of Port Moody at present is that it is far less polluted than one might anticipate under the circumstances. Although the gross aggregate of pollutant entering into Port Moody is unknown, it must constitute a considerable volume of fairly heavily-loaded effluent. The organic constituents alone might be expected to reduce the dissolved oxygen levels to much lower concentrations than were present at the time of our more recent surveys. Although the dissolved oxygen levels in the bottom water have been usually quite low, they have not prevented the fish and invertebrates, particularly the crabs, from continuing to survive. The petroleum refineries have contributed considerable amounts of effluent. Apparently this has not been very harmful to the fish life, up to the present time. Oil films have often been observed in large slicks in Port Moody, but they appear to dissipate rapidly on the ebb tide. Phenol concentrations have been high only in the surface films of oil and comparatively low in subsurface waters

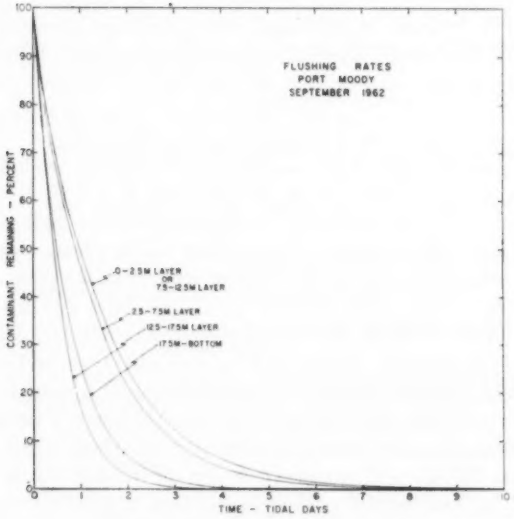

FIG. 10. Flushing curves for various depth intervals, from current data of September 1962.

even at a depth of 1 m. One might conclude that while the long-term effects of settling solid wastes may be quite devastating eventually to the bottom organisms in the inlet, the effects of dissolved, water-borne wastes do not seem to have been startlingly severe.

There have been a considerable number of studies made of estuaries and inlets for the purpose of evaluating flushing rates in areas where there is a substantial amount of run-off from rivers. Important among these studies are the works of Ketchum (1951), Ketchum and Keen (1953), and Tully (1949). In every case, fresh water could be used as a tracer for estimating the rate of flushing, and in some cases there was intensive vertical mixing. Cline and Fisher (MS, 1959) reviewed the various methods for estimating the flushing time of tidal estuaries with special application to the James River. Gibson (1959) critically analyzed the problem of estimating flushing time in estuaries and tidal embayments. He developed by theoretical considerations a relationship between the tidal prism and the total volume of a bay with no run-off to arrive at the exchange ratio. The average exchange ratio was given as the ratio of the tidal prism volume to the total high tide volume of the embayment. For the whole volume of Port Moody the exchange rate, as determined here, is essentially the same as that given by Gibson's approach.

In any study of flushing, one is always faced with the problem of the amount of mixing that occurs in a system. In Port Moody, which is highly stratified, the amount of vertical mixing is relatively small. Rather than segment the inlet by horizontal sections from the entrance to the head, as done by Ketchum (1951), it seems more realistic to segment the bay vertically. With current measurements at a number of discrete depths, one at least has the opportunity of determining the amount of exchange that occurs in different layers of the bay. This does not necessarily eliminate the problem of mixing in that particular depth interval or solve the problem of re-entry of effluent that left on the previous ebb.

In Port Moody, which has a length of 3.5 nautical miles (6.5 km), the tidal excursion may actually approach the full length of the bay. For an average current velocity of 20 cm/sec during a tidal fall of 6.2 hr, for example, the distance travelled by a particle would be about 4.5 km or 73% of the length of Port Moody. Thus, a large part of the surface water present in the inlet at a particular time could be expected to be removed in most part on a large ebb tide. As this water leaves Port Moody and enters the Burrard Inlet–Indian Arm system, it becomes swept downstream with the much more rapid surface flow of water in this system. There is a greater ebb bias in Indian Arm and Burrard Inlet than in Port Moody, because of the relatively large volume of fresh water that flows from the Indian River into the surface waters at the head of Indian Arm. Therefore, it can be expected that the water removed on a given ebb tide from Port Moody will not likely return on the next flood, in the surface layer. Because of the inward bias of deep-water flow in the Burrard Inlet–Indian Arm system, there could conceivably be a return of the effluent from Port Moody on the next flood in the deep water. However, it would be very highly

diluted with the comparatively unpolluted water and in its return would not constitute a pollution hazard. It could be concluded from this reasoning, therefore, that as long as the Burrard Inlet–Indian Arm system is maintained relatively pollution-free, the waters of Port Moody, with the rapid rate of tidal flushing, would not be expected to become highly polluted unless the input of wastes was considerably increased. The long-term cumulative effects, of course, may create increasingly more undesirable conditions along the bottom.

One of the first aspects of the water quality in Port Moody which may deteriorate to intolerable levels is the dissolved oxygen at intermediate depth. The oxygen minimum in this layer appears to be associated with a zone of stagnation. It may be possible with good seasonal data eventually to calculate the rate of oxygen utilization in this zone, following the method used by Barnes and Collias (1958) for basins in Puget Sound. Once the rate of utilization exceeds replenishment on an annual basis, a steady decline of dissolved oxygen concentrations in Port Moody can be anticipated.

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Diurnal and Seasonal Changes in Distribution of Two Planktonic Ostracods, *Conchoecia elegans* and *Conchoecia alata minor*[1,2]

By R. A. McHardy[3] and B. McK. Bary[4]

ABSTRACT

Diurnal and seasonal changes in the distributions of two planktonic ostracods, *Conchoecia elegans* Sars and *C. alata minor* McHardy, have been considered in relation to changes in the temperature, salinity, oxygen concentration, and subsurface light in Indian Arm, British Columbia. Both species occurred frequently throughout the water column except near the surface. There is the suggestion (reinforced by data from other B.C. inlets) of an upper temperature limit for *C. alata minor*, which is lower than for *C. elegans*. Salinity and dissolved oxygen environments appear to have been similar for the two species. An upinlet displacement of both species-populations which took place in the early months of the year could possibly have been initiated by water which had intruded into the depths of Indian Arm. The vertical distributions of the two species showed statistically significant changes which varied with time of day, season, and geographic position. It was not possible to show that the depth of daylight distribution was strongly related to the penetration of light.

INTRODUCTION

In earlier works, the marine ostracods received much taxonomic consideration but information on their ecology was scanty (Davidson, 1924; Elofson, 1941; Jesperson, 1923). Recent investigations have concentrated on the ecology of marine ostracods, more especially those of the benthos (Benson, 1959; Kornicker, 1958, 1961, 1963; Kornicker and Wise, 1960), but little information is available on the ecology of marine ostracods of the plankton. An opportunity to add to this latter field became available with the discovery of an abundance of ostracods in the plankton of an easily accessible fjord, Indian Arm, British Columbia.

Previously, Indian Arm was investigated with regard to the cyclic changes in its physical oceanography (Gilmartin, 1962) and its primary productivity (Gilmartin, 1964). The present study is concerned with seasonal and diurnal changes found to occur during 1960 in the distributions of the planktonic ostracods, *Conchoecia elegans* Sars and *C. alata minor* McHardy (1964a), in the inlet, and is the first contribution toward a knowledge of the distribution of zooplankton in the Inlet.

FIELD METHODS

Indian Arm (Fig. 1) may be regarded as similar in physiography to many British Columbia inlets. Its length is about 22 km, its average width slightly

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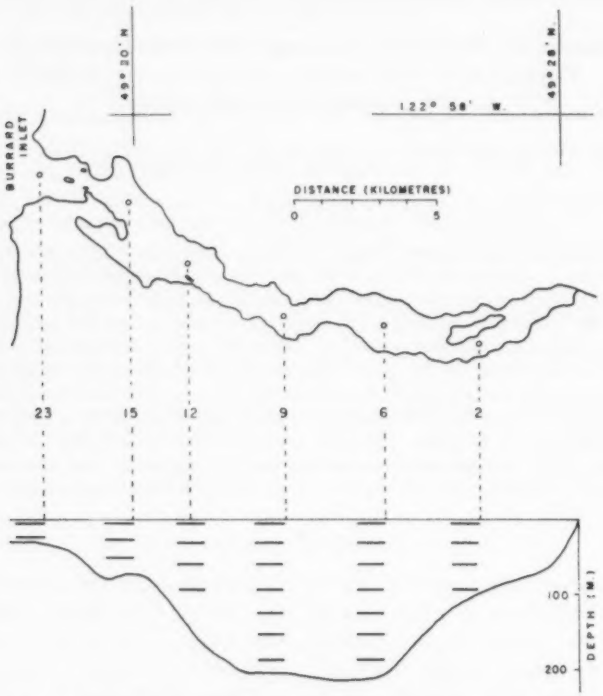

FIG. 1. Indian Arm, plan view and longitudinal profile showing oceanographic stations (numbered) and paths of plankton tows (bars on profile).

more than 1 km, and its depth is about 200 m over the middle three-quarters of its length and about 30 m over the sill at its mouth. The sill depth is shallower than average for B.C. inlets, but is not the shallowest (Gilmartin, 1962). Oceanographic properties of Indian Arm are controlled by addition of fresh water through river and peripheral stream runoff, and precipitation; and by intrusion of saline water from, and loss of brackish near-surface water into the Strait of Georgia, by way of Burrard Inlet (Gilmartin, 1962).

Plankton samples plus temperature and salinity data were collected near midnight and noon from stations 2, 6, 9, and 12 (Fig. 1) on January 6 and 7, March 23 and 24, May 26 and 27, July 26 and 27, September 7 and 8, and November 1 and 2; oxygen concentrations were measured only for station 6 and light data were observed only at noon. Additional information was obtained from plankton collections taken from stations 15 and 23 during some of the stronger tidal movements and from temperatures and salinities for the months of February and May (complete oceanographic data are recorded in Anon, MS, 1961). Supplementary information was obtained from plankton collections and oceanographic data gathered in other B.C. inlets.

Water samples and temperatures were collected from standard depths. Temperature was recorded with reversing thermometers; salinities and oxygen concentrations were determined by the standard Mohr and Winkler methods, respectively (Strickland and Parsons, 1961). Subsurface light was measured with a Weston photocell, which Strickland (1958) estimates has a limit of sensitivity of 10^{-4} langlies/min; the instrument used was calibrated against an Epply pyrheliometer. It was lowered to ½ m below the surface and then by 5-m intervals from the surface to the depth of no detectable light (i.e. to a depth with a value less than 10^{-4} ly/min).

Clarke-Bumpus plankton samplers (Clarke and Bumpus, 1950), modified according to Paquette and Frolander (1956) and equipped with nets of 55 meshes per inch (0.40-mm mesh size), were towed horizontally in series for 15 min, over a distance of about 1 km at a speed of about 2.4 knots. Plankton samples were collected at vertical intervals of 30 m from surface to bottom except at the shallow stations (15 and 23) where intervals were 25 and 20 m (Fig. 1, profile). The volume of sea water filtered (8–10 m^3) was calculated using a calibration value of 40 litres/count, where one count represented 10 revolutions of the flowmeter impellor (Yentsch and Duxbury, 1956; McHardy, 1961). For samples from station 15 and 23, all specimens were examined. From other stations, a representative 10% of each sample was withdrawn using a vacuum-assisted subsampler (McHardy, 1964b).

OCEANOGRAPHIC FACTORS AND LIGHT

Gilmartin (1962) studied the physical oceanography of Indian Arm from September 1956 to October 1959. He showed that intrusions of water of relatively low temperature, high salinity, and high oxygen concentration penetrated the depths of the inlet during the winters of 1957 and 1959. In the early months of 1960, changes in oceanographic properties of Indian Arm similar to those described by Gilmartin indicated that another intrusion was occurring.

During 1960, the largest vertical temperature gradient of Indian Arm water was within the upper 10 m; the range fluctuated seasonally from less than 1 C° in winter to as much as 10 C° in summer. Below 10 m the gradient varied seasonally within a range of 5 C°, roughly between 7 and 12°C (Fig. 2). The temperature in the deeper water fell by more than 0.5 C° during the period from January to March, mostly from February to March. Mixing between this deep, relatively low-temperature water and the warmer water in the inlet was apparent by May. This mixing progressed upinlet, as shown by the upinlet direction of the gradual leveling of isotherms (e.g. the 7.6°C isotherm, Fig. 2). Because of its low salinity, water at the surface in Indian Arm, even when cold, tends not to sink. The cold water that appeared in the depths, therefore, must have originated outside Indian Arm.

The gradient of salinity was largest in the upper 10 m; it could be as little as 5‰ or as much as 15‰. For water deeper than 10 m the salinity increased a few parts per thousand. Below 75 m it usually approximated 27‰, about which it varied for the deeper waters during 1960 (Fig. 3). Along the bottom

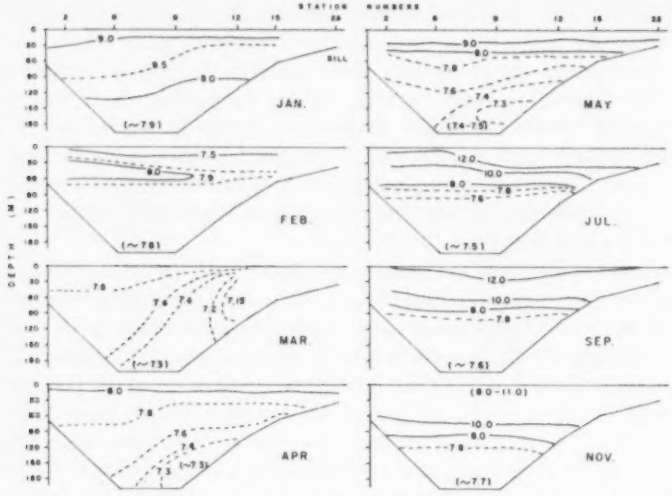

FIG. 2. Isotherms (°C) of Indian Arm for 1960 (those in thermocline largely omitted).

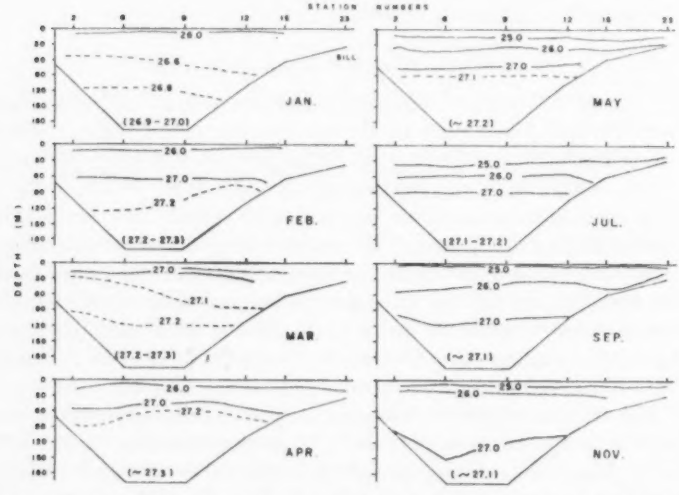

FIG. 3. Isohalines (‰) of Indian Arm for 1960 (those in halocline largely omitted).

of the deep basin, salinity increased from about 27.0‰ in January to approximately 27.3‰ by April and decreased gradually to near 27.1‰ by November. In January, the 27.0‰ isohaline was close to the bottom (at about 200 m); by February it was located at about 65 m, and by March at approximately 15 m. During later months it returned to greater depths, and was located between

100 and 150 m in November (Fig. 3). Since precipitation exceeds evaporation in Indian Arm (Gilmartin, 1962) and an *in-situ* increase in salinity is not reasonable, the increase in salinity undoubtedly originated outside the inlet.

In 1960, at station 6, oxygen concentration at the surface was always greater than 10 mg/litre (Fig. 4). From May to September it exceeded saturation (Richards and Corwin, 1956), probably because of the high rate of photosynthesis that occurs in Indian Arm during these months (Gilmartin, 1964). Oxygen concentrations, each weighted according to the span of depth it represented, are averaged for shallow (0–65-m), intermediate (65–125-m), and deep (125–200-m) intervals, in order to illustrate the magnitude of seasonal changes within the water column (Fig. 4). From January to February, oxygen concentration increased considerably in the intermediate and the deep intervals (3.5–6.0 and 2.0–6.0 mg/litre, respectively), but decreased in the shallow interval (7.0–5.4 mg/litre). The increase at deeper levels accompanied the previously mentioned decrease in temperature and increase in salinity.

It seems that the oceanographic changes discussed above constitute evidence for an intrusion of water similar to those reported by Gilmartin (1962). It is evident that the greater part of the intrusion of 1960 occurred

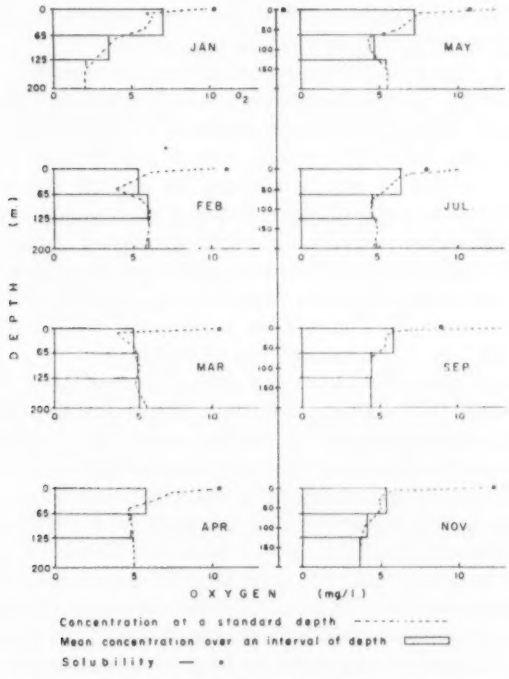

FIG. 4. Dissolved oxygen, vertical distribution at station 6, Indian Arm, for 1960.

between January and March (Fig. 2–4), but since the sampling was only once a month, neither the frequency of intrusion nor the amount of water intruded at a time in this period is known. There is evidence that by July the intruded water was beginning to be integrated and during the months that followed, it gradually lost its distinctiveness through mixing with the resident water (Fig. 2–4). The processes can be followed from an initial raising towards the surface of once-deep isolines in the downinlet position (February–March), their gradual leveling (May–July) and then their subsequent sinking.

DISTRIBUTION OF OSTRACODS

Distributions of *Conchoecia elegans* (Fig. 5) and *C. alata minor* (Fig. 6) are presented on longitudinal profiles of Indian Arm as areas of negligible, moderate, and high concentrations (< 0.1, 0.1–0.9, > 0.9 specimens/40 litres, respectively).[5]

The edge of the area of moderate concentration of *C. elegans* (Fig. 5) often approached but never reached the surface. Only two specimens were obtained from about 70 surface samples. The main region of high concentration of the population of *C. elegans* moved from downinlet in January and March to upinlet in May. The region of moderate concentration followed the movement of the highest concentration upinlet in May, but the concentrations became more uniformly spread in July, September, and November.

Specimens of *Conchoecia alata minor* occurred frequently in samples from 30 m and deeper, but at the surface they were present in only two of the 70 samples examined. Moderate concentrations of *C. alata minor* occupied intermediate depths while high concentrations were usually deep (Fig. 6). In July and September, patches of high concentration existed also at lesser depths. In January and March, zones of high concentration were moderately prominent upinlet, and in May there was a pronounced movement away from the downinlet position. By July there was a more or less even distribution of the zone of high concentration along the basin, which by September and November had moved slightly downinlet. The upinlet movement for this species was less extensive than that for *C. elegans* (cf. Fig. 5 and 6).

The intrusion into Indian Arm during the early months of the year of water of low temperature, high salinity, and high oxygen concentration, may have initiated the upinlet movements of the two populations. Later in the year they spread toward the downinlet position, apparently in coincidence with the period during which the oceanographic properties returned to a more uniform distribution. The correlation between the intrusion from outside and the upinlet movement of ostracods, however, is not close. A comparison of Fig. 2 and 3 with Fig. 5 and 6 indicates that the movement of ostracods occurred somewhat later than the main period of intrusion. If there were some oceanographic factor that served to repel the species from downinlet, it could, perhaps, have accumulated during the period of the intrusion or entered Indian Arm late in the period.

[5]Specimens/40 litres = specimens/count of flow meter of sampler.

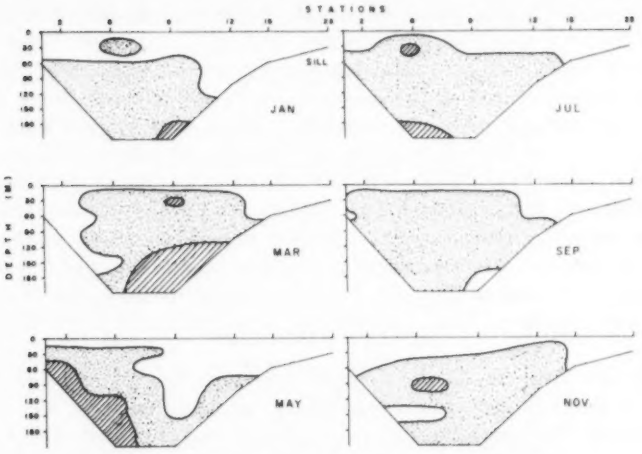

FIG. 5. *Conchoecia elegans* Sars, longitudinal distribution in Indian Arm for 1960 (from night collections only). Concentrations of specimens/40 litres: < 0.1 (clear), 0.1–0.9 (stippled), and > 0.9 (hatched).

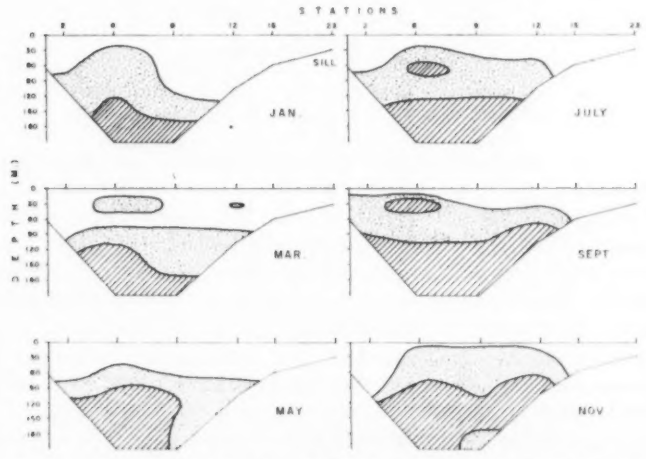

FIG. 6. *Conchoecia alata minor* McHardy, longitudinal distribution in Indian Arm for 1960 (from night collections only). Concentrations as specimens/40 litres: < 0.1 (clear), 0.1–0.9 (stippled), and > 0.9 (hatched).

In either case, the effectiveness of this factor would seem to have been maintained (to judge from the continued upinlet location of specimens) until reduced by dilution pursuant on mixing with the resident water. This mixing seems to have been well advanced by July and, by this time, both populations of ostracods were spread downinlet.

Toward the sill, at stations 15 and 23, concentrations of *Conchoecia elegans* were less than 0.2 specimen/40 litres and those of *C. alata minor*, less than 0.05 specimen/40 litres. Probably these low numbers occurred because the maximal depths at station 15 (60 m to bottom) and 23 (30 m to bottom) tended to approach the minimal depths attained by the specimens in the water column. As is demonstrated later in relation to vertical migration (Fig. 11 and 12), these minimal depths reached are less at night and less for *C. elegans*. Although such a shallower distribution for *C. elegans* should favour the possibility of specimens becoming entrained in outflowing water, realisation of this appears not to have occurred. Few specimens of this species, and fewer of *C. alata minor*, were found over the sill (Fig. 7). Possibly, therefore, exchange of specimens over the sill may be of little importance in maintenance of the Indian Arm population.

In Indian Arm, moderate to high concentrations of *Conchoecia elegans* occurred at temperatures from 7.3 to 12.0°C, at salinities from 24.6 to 27.3‰, and at oxygen concentrations from roughly 2 to 7 mg/litre. For equivalent concentrations of *C. alata minor*, the limits were 7.3–10.8°C, 24.9–27.3‰, and roughly 2–7 mg/litre. It appears as though in Indian Arm the upper limit for temperatures is lower for *C. alata minor* than for *C. elegans*. This suggestion is supported by supplementary data collected in other B.C. inlets. (Fig. 8).

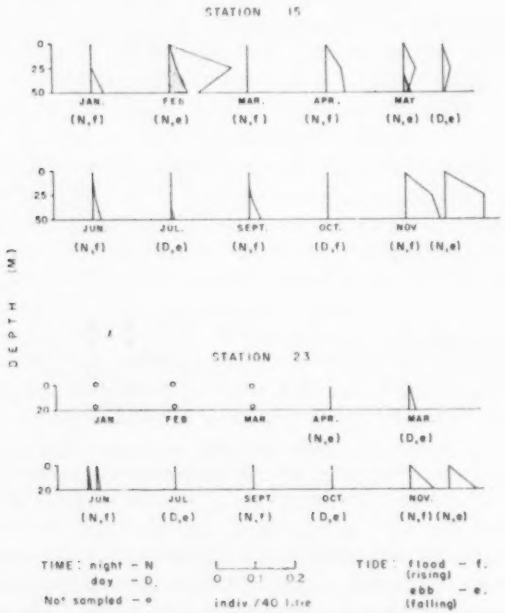

FIG. 7. Abundance of *Conchoecia elegans* Sars (unshaded) and *C. alata minor* McHardy (shaded) at stations 15 and 23 during some of the stronger tidal movements in 1960.

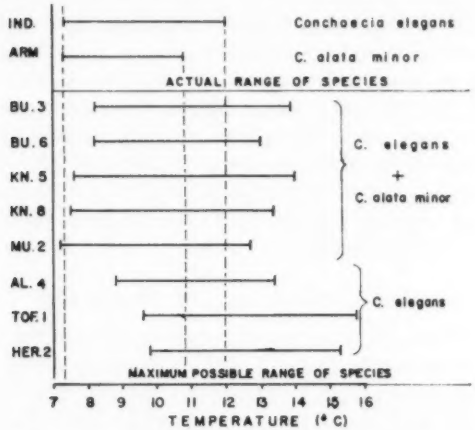

FIG. 8. Temperature ranges of specimens of *Conchoecia elegans* Sars and *C. alata minor* McHardy in Indian Arm compared with temperature ranges of the water columns from which specimens of one or both species were taken in other B.C. inlets. Locations:

Bute (BU)	(3) — 50°32′N, 125°00′W
	(6) — 50°46′N, 124°54′W
Knight (KN)	(5) — 50°42′N, 125°49′W
	(8) — 50°53′N, 125°39′W
Muchalat (MU)	(2) — 49°40′N, 126°08′W
Alberni (AL)	(4) — 49°03′N, 124°51′W
Tofino (TOF)	(1) — 49°12′N, 125°39′W
Herbert (HER)	(2) — 49°21′N, 125°57′W

Bottom to surface hauls with an open 1-m net revealed that both species existed in Bute, Knight, and Muchalat Inlets but only *Conchoecia elegans* occurred in Herbert, Tofino, and Alberni Inlets (Fig. 8). Oceanographic data collected concurrently (Anon., MS, 1959) showed that salinity and oxygen concentrations in these inlets were probably not limiting since both species lived in Muchalat Inlet where the extremes of these factors in the water column approximately encompassed those found in the other inlets (2.2–33.1‰ and 0.3–11.62 mg/litre). Temperature, on the other hand, seems to have differed among inlets such that those inlets deficient in low-temperature water did not maintain *C. alata minor*. In Bute, Knight, and Muchalat Inlets, where both species were present, temperatures ranged well into those low values in which moderate to high concentrations of both species occurred in Indian Arm. On the contrary, in Herbert, Tofino, and Alberni Inlets, where only *C. elegans* was collected, the temperature did not extend to the lower values which appear to be conducive to the presence of *C. alata minor*. The evidence suggests that *C. alata minor* was favoured by the lower temperatures and could not exist at the higher temperatures in those inlets from which only *C. elegans* was collected. Perhaps the upper temperature limit for *C. alata minor* lies in the vicinity of 10.8°C, the highest at which moderate concentrations were present in Indian Arm.

Jesperson (1923) recorded *Conchoecia elegans* as having been collected at temperatures below zero and at salinities greater than 34‰. Elofson (1941) reported the species from temperatures of −1.49 to 23.5°C and salinities of 19.96 to over 35‰. He concluded that *C. elegans* was an eurythermal, euryhaline species. *Conchoecia alata major* Rudjakov,[6] a form closely related to *C. alata minor*, was collected at the Ocean Weather Station "P" (50°N, 145°W) from a single vertical haul between 1250 m and the surface (McHardy, 1964a), where temperature ranged between 2.6 and 12.2°C and salinity lay between 32.7 and 34.5‰ (Tabata *et al.*, MS, 1960).

VERTICAL MIGRATION OF OSTRACODS

To investigate changes in the vertical distributions of the two species, data were examined with reference to two positions (stations 6 and 9), seven depths (30-m intervals), six seasonal periods (January, March, May, July, September, and November), and two times of day (midnight and noon). Each concentration, x specimens per 40 litres, was transformed to $\sqrt{100\,x + 1}$ so that the data would be more manageable for plotting (Fig. 9 and 10) and more stable in variance for

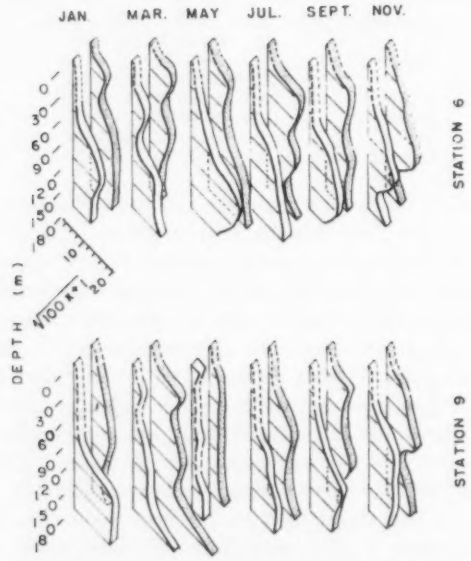

FIG. 9. Vertical distribution of *Conchoecia elegans* Sars, abundance of specimens as $\sqrt{100x + 1}$ (where x = specimens/40 litres) in relation to depth, time of day (curve with white edge = noon; with shaded edge = midnight), season, and position (station).

[6]Specimens from Station "P" were compared with specimens of *C. alata major* donated by Dr Rudjakov.

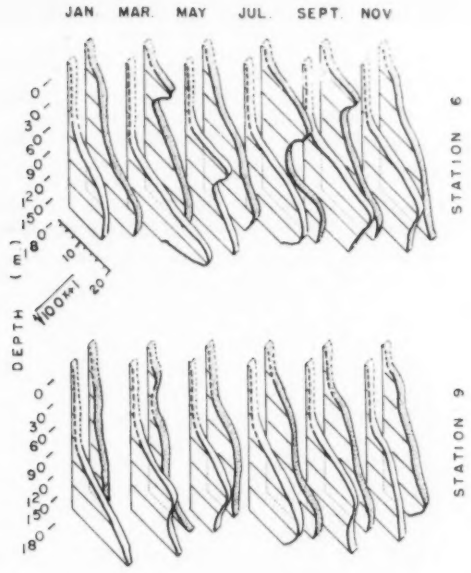

FIG. 10. Vertical distribution of *Conchoecia alata minor* McHardy, abundance of specimens as $\sqrt{100x+1}$ (where x = specimens/40 litres) in relation to depth, time of day (curve with white edge = noon; with shaded edge = midnight), season, and position (station).

subsequent analysis of variance (Table I). The three-dimensional presentations in Fig. 9 and 10 illustrate how the abundance of specimens varies with depth, time of day, season, and position; the analyses of variance in Table I show the degree to which these factors are effective in determining differences in abundance. To show statistical significance for diurnal change in the depth distribution, for instance, the F-value for interaction between depth and time of day (D × T) would have to exceed that which may occur by chance ($p < .05$ or .01). Discussion of these results follows under General Analysis.

The extent of variation occurring at different levels has been measured by examining the differences in depth exhibited by quartiles of the vertical distribution, where the shallowest quartile is considered the first, the intermediate one is termed the second, and the deep one the third. The depth of each quartile is illustrated (Fig. 11 and 12) and examined by analysis of variance (Table II) with reference to the above-mentioned two positions, six seasons, and two times of day. Discussion of this approach and consideration of the possible effect of light penetration on the daylight depths of quartiles follows under Analyses of Quartiles.

TABLE I. Analyses of variance for the effects of depth, time of day, season, and position on the abundance of *Conchoecia elegans* and on the abundance of *C. alata minor*.

Source	Degrees of freedom	*C. elegans* Mean square	*C. alata minor* Mean square
Depth (D)	6	174.5**	753.0**
Time (T)	1	15.9	13.5
Season (S)	5	9.3*	47.8**
Position (P)	1	5.6	231.2**
D × T	6	47.3**	52.6**
D × S	30	8.3**	10.8**
D × P	6	6.4	16.0**
T × S	5	3.1	11.3
T × P	1	2.8	36.3**
S × P	5	61.9**	1.3
D × T × S	30	2.4	8.4**
D × T × P	6	1.5	7.8
D × S × P	30	8.7**	7.0**
T × S × P	5	6.7	9.6
Means:	37		
Total:	167		
Error:	30	4.3	5.3

GENERAL ANALYSIS

For *Conchoecia elegans*, statistically significant variations are shown for depth and for interactions between depth and time, between depth and season, and among depth, season, and position (Table I). From these results it may be interpreted that abundance of specimens varied with depth ("depth" being a significant source, Table I) in a manner which differed according to time of day (D × T interaction) and that this variation with depth altered with season in a manner which differed between positions (D × S × P interaction). The analysis, as well as the illustration, shows that diurnal variation in vertical distribution was not measurably affected by season or position (D × T × S and D × T × P interactions not significant). The fact that time of day (representing

TABLE II. Analyses of variance for the effects of time of day, season, and position on the depth of quartiles of the vertical distribution of *Conchoecia elegans* and of *C. alata minor*.

		C. elegans			*C. alata minor*		
		Mean Square					
Source	Degrees of freedom	1st quartile	2nd quartile	3rd quartile	1st quartile	2nd quartile	3rd quartile
Time (T)	1	13,585**	3,220.2**	693.4	219.0	201.2	201.3
Season (S)	5	439	837.5*	445.8	967.9*	459.8*	136.5
Position (P)	1	210.1	793.5*	187.0	931.3	364.2	102.1
T × S	5	622.9	529.1*	380.6	241.2	39.6	26.0
T × P	1	63.4	32.6	222.0	243.8	1.3	7.5
S × P	5	176.8	239.4	323.8	96.5	114.0	65.2
Error:	5	239.3	100.0	226.5	163.1	65.5	240.6

summing of all the day values and all the night values irrespective of depth, season, or position) is not shown to be a significant source of variation indicates that the data were relatively unbiased by possible daylight escapement.

According to Table I, overall abundance changed seasonally in a manner which differed between positions (S × P interaction). This change reflects the concurrent increase at station 6 and decrease at station 9 which appeared in May as part of the mass upinlet movement of this species between March and May (see Distribution of Ostracods).

For *Conchoecia alata minor*, significant variations are shown for depth; for interactions between depth and time of day, depth and season, and depth and position; as well as for the interactions among depth, time, and season, and among depth, season, and position (Table I). Other significant variations are attributed to position, to interaction between position and time, and to season. Thus, the variation with depth is interpreted to be altered according to time of day (D × T interaction), season (D × S), and position (D × P); the already-mentioned alteration due to time of day is considered to be influenced by season (D × T × S interaction); and the change due to season is understood to be affected by position (D × S × P). It may be concluded from the analysis from Fig. 10, that the vertical distribution of this species is determined by factors of seasonal and positional as well as diurnal fluctuation.

The analysis (Table I) also suggests that overall abundance changed seasonally and that relative abundance between positions differed according

to time of day. An increase in abundance from January on through to the slight decrease in November (Fig. 10) explains the interpretation regarding season. The interaction between time of day and position could possibly have been due to a sampling error where a lag between collecting at the two positions (approximately 1 hr) caused diurnally-migrating specimens, recognized at a certain sampling depth at one position, to have escaped discovery by migrating just below this depth, and between sampling depths, at the other position. The fact that the difference between positions is not shown to have varied significantly with season (S × P interaction), is in accord with the observation that movement up and down the inlet was relatively slight between these stations for this species (see Distribution of Ostracods).

Analyses of Quartiles

For *Conchoecia elegans*, the depths of the first quartile (upper level) are shown (Table II) to have had highly significant ($p < .01$) variation with time of day; those at the second quartile exhibited less highly significant variation with time, as well as lesser significance ($p < .05$) with season, position, and

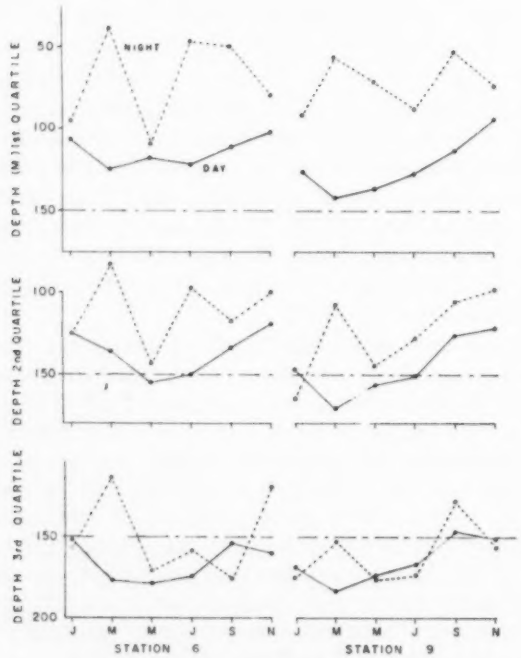

FIG. 11. Diurnal vertical migration of *Conchoecia elegans* Sars, depths of quartiles for the vertical distribution at stations 6 and 9, Indian Arm, for 1960.

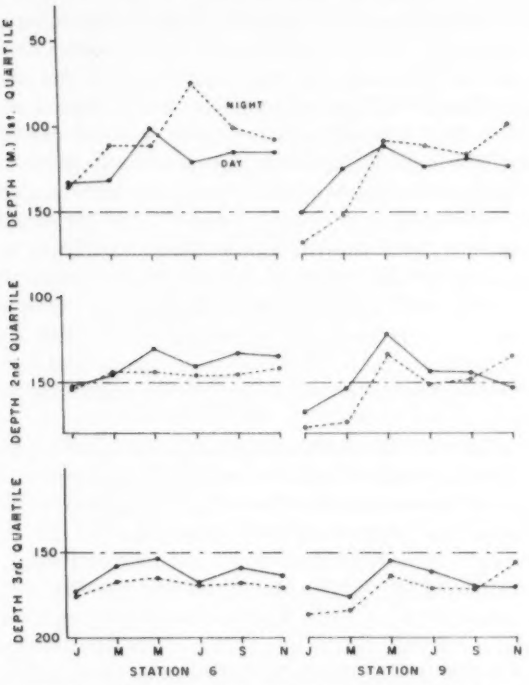

FIG. 12. Diurnal vertical migration of *Conchoecia alata minor* McHardy, depths of quartiles for the vertical distribution at stations 6 and 9, Indian Arm, for 1960.

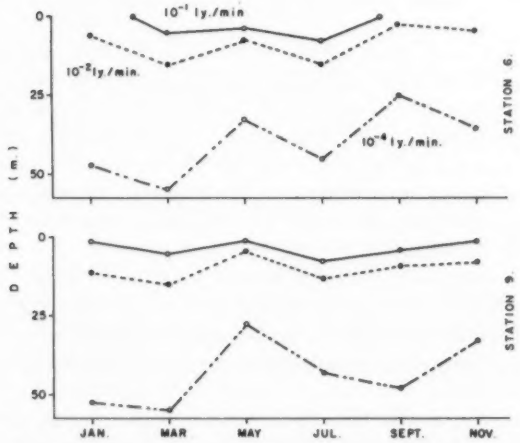

FIG. 13. Depth of penetration of light (langlies/minute) at the times of daylight plankton collections at stations 6 and 9, Indian Arm, for 1960.

interaction between time and season; while those at the third quartile showed no significant variation. The effect of time of day shows vertical migration to be diurnal and the interaction of time and season shows diurnal migration at the second quartile to be influenced by season. In addition, season and position are shown to have had different effects on the vertical displacement of this second quartile. The diurnal influence appears, from the analysis, to have diminished in significance from shallow to deep quartiles, as if the causal agent also diminished with depth. For *C. alata minor* (Table II), the only significant factor appears to have been season, which caused significant variation in the vertical distribution of the first and second quartiles, but not the third. Although this analysis has been useful, it is rather weak because it involves the quartile which in this case is interpolated and represents a reduction (by seven depths to three) of information. Such weakness is demonstrated by the fact that many effects shown to be significant in the general analysis are masked in this one for quartiles.

The depths of quartiles at midday appear to form certain trends (Fig. 11 and 12, *day*). To test whether there might be a relationship between these suggested trends and the trend shown for the penetration of light (Fig. 13, 10^{-4} ly/min), a correlation analysis was performed. This analysis showed that any relationship with light that might have existed for the daylight distribution of *Conchoecia elegans* was not statistically significant for the first, second, or third quartiles ($r = 0.13$, 0.17, and 0.003; $p < .05$). For *C. alata minor*, significant correlation was shown for the second quartile ($r = 0.72$; $p < .01$), but not for the first and third quartiles ($r = 0.27$ and 0.57; $p < .05$). Whether the significant correlation above expresses an actual relationship or a chance (< 1 in 100) coincidence, is unknown. In either case, the evidence at hand does not justify any estimation as to its biological meaning.

SUMMARY

1. In Indian Arm both species often occurred in moderate to high concentrations at 30 m and below in water having salinities from 25 to 27‰ and oxygen concentrations from 2 to 7 mg/litre. These concentrations of *Conchoecia elegans* occurred at temperatures between 7.3 and 12.0°C, whereas those of *C. alata minor* existed between 7.3 and 10.8°C. Some B.C. inlets deficient in low-temperature water apparently can maintain *C. elegans* but not *C. alata minor*. It is suggested that the upper limit of temperature for *C. alata minor* is lower than for *C. elegans*.

2. Upinlet movement of the population which occurred during the early months of the year for *Conchoecia elegans* and, to a lesser extent, for *C. alata minor*, may have been related to an intrusion of water of low temperature, high salinity, and high oxygen content that entered the depths of Indian Arm from outside the inlet.

3. Populations of the two species were more or less restricted within Indian Arm, probably because of the shallowness of the sill (30 m). Within this restriction, *Conchoecia elegans* had the greater dispersal, vertically and horizontally.

4. Specimens of *Conchoecia elegans* showed a statistically significant seasonal change in vertical distribution influenced by geographic position, and a significant diurnal vertical migration not measurably affected by either position or season, but having a tendency to diminish with increased depth (from the shallow to the deep quartiles of the vertical distribution).

5. Specimens of *Conchoecia alata minor* showed vertical migrations which were significantly altered by time of day, season, and geographic position. The diurnal change in vertical distribution was influenced by season and the seasonal change in vertical distribution was affected by position.

6. An attempt to relate the daylight depths of quartiles of the vertical distributions with the penetration of light was not convincing.

ACKNOWLEDGMENTS

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Distribution and Morphology of the Medusa, *Calycopsis nematophora*, from the North Pacific Ocean[1]

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ABSTRACT

The medusa, *Calycopsis nematophora*, is reported from the North Pacific for the first time since its original description. It is restricted to arctic-subarctic surface waters. Radial canal variation and other morphological characters are discussed.

INTRODUCTION

Ten species are generally recognized in the anthomedusan genus *Calycopsis* Fewkes. This world-wide genus shows geographic speciation, but most species are known from few localities. Also, due to the morphological variation of characters (such as radial canals) a few forms were described as new species or doubtfully identified as a previously described species. These were probably variants of known species.

Three species of *Calycopsis* have been reported from the Pacific Ocean from only a few captures in widely separated areas: *C. borchgrevinki* (Browne) from the Pacific subantarctic, *C. simulans* (Bigelow) from the tropical Pacific, and *C. nematophora* Bigelow from the arctic-subarctic. *Calycopsis nematophora* has not been reported since its original description by Bigelow in 1913. This paper is a report on *C. nematophora* collected in Pacific waters during 1961–63. Specimens collected over a large geographic area show morphological variations in relation to their distribution.

MATERIAL AND METHODS

A total of 661 plankton samples from 67 stations was examined for *Calycopsis*: 215 from the subarctic North Pacific including the Bering Sea, 431 from 16 stations off the Oregon coast, and 15 from the northeastern central Pacific (Fig. 1). The samples from the Oregon coast were collected with a modified 6-ft Isaacs-Kidd midwater trawl towed at 6 knots. The other samples were collected with a 3-ft modified Isaacs-Kidd midwater trawl towed at 4 knots. All tows were oblique, but varied in depth. Most of the tows off the Oregon coast were 0–200 m; a few were 0–1000 m. Of the remaining tows, ten were 0–500 m and the rest 0–200 m or shallower. The tows in which specimens were captured are described in Table I.

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TABLE I. Tows in which *C. nematophora* were captured.

Date	Latitude (*N*)	Longitude (*W*)	Range of depth of tows (*m*)	Number of specimens
1961				
May 4	55°00′	155°00′	0–50	1
14	52°00′	166°20′	0–50	1
June 1	50°00′	177°00′	0–50	1
1	49°00′	176°30′	0–50	1
10	53°02′	174°37′	0–50	3
12	53°28′	170°01′	0–50	2
13	54°20′	170°00′	0–50	1
13	55°09′	170°00′	0–50	1
23	58°02′	180°00′	0–150	2
24	58°04′	180°00′	0–100	1
24	58°07′	180°00′	0–25	9
24	58°12′	179°57′	0	1
24	53°26′	169°57′	0–50	2
July 10	51°18′	176°50′	0	1
12	49°57′	178°09′	0–50	1
Aug. 4	51°02′	175°27′	0–125	1
4	51°02′	175°27′	0–150	1
4	51°02′	175°27′	0–75	1
Nov. 7	44°39′	125°32′	0–200	1
15	46°14′	125°15′	0–200	1
1962				
Jan. 9	44°39′	126°59′	0–290	1
Sept. 3	49°50′	160°50′	0–100	1
4	49°50′	160°50′	0–250	3
4	49°46′	160°48′	0–500	1
1963				
Sept. 20	44°39′	127°00′	0–200	1

DISTRIBUTION

Although Bigelow (1913) considered *C. nematophora* a surface form restricted to the Bering Sea region, the medusa appears to be epipelagic with a wider distribution. Its distribution (Fig. 1) agrees with the general surface water movements in the subarctic as outlined by Uda (1962). Of the 40 individuals of *C. nematophora* collected, 36 were obtained north of 49°N, and the other four were obtained off the Oregon coast. The occurrence of *C. namatophora* off the Oregon coast suggests expatriation from more northern latitudes, probably due to the West Wind Drift and the California Current system.

Only 11 of the 40 specimens were obtained from tows 100 m or deeper (Table I). This suggests that *C. namatophora* inhabits surface waters where its distribution is influenced by the surface water movements.

MORPHOLOGY

Bigelow (1913) presented an excellent description of *C. nematophora* from 29 specimens collected at nine locations (Fig. 1). He stated that the medusae are higher than broad, except when contracted. The medusae examined in this study are contracted and are almost as broad as high (1 or 2 mm higher than broad). The margin of the bell cavity is withdrawn, turned inward and upward.

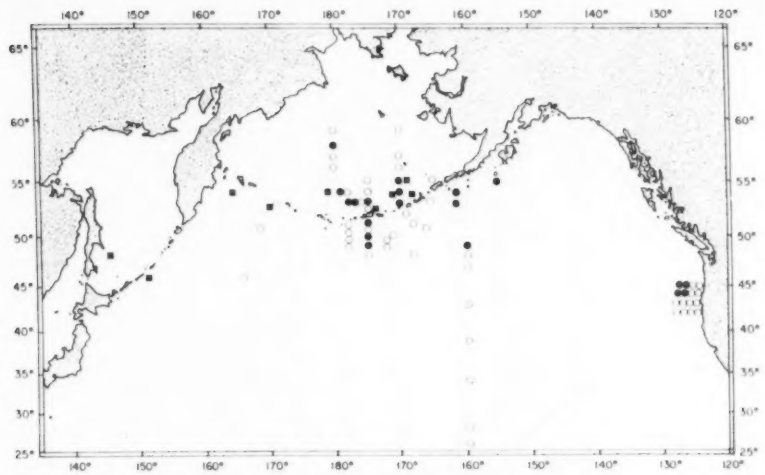

FIG. 1. Distribution of samples and of *Calycopsis nematophora*. Solid circles, *C. nematophora* present; open circles, *C. nematophora* absent; solid squares, *C. nematophora* reported by Bigelow in 1913.

The height ranges from 7 to 30 mm, with most of the specimens at least 20 mm high. As a rule, the larger the specimen, the thicker the gelatinous material of the bell. The jelly is thin and flexible in the three smallest specimens (7, 10, and 12 mm), while all of the larger specimens are rigid and have fairly thick jelly. The larger specimens also display the slight apical depression mentioned by Bigelow. This depression appears to be an adult characteristic of this species.

All but one of Bigelow's specimens were compressed either radially or interradially. Only seven of the recent collection are compressed, probably by preservation. Five live specimens observed on board the U. S. Coast and Geodetic Survey ship *Pioneer*, showed no compression.

The manubrium is only about one-fourth the length of the bell cavity in preserved specimens (Fig. 2), and one-third in live animals. In preserved specimens the lips of the manubrium are complexly folded, turned upward and out, while in the live animals they extend downward. Labial nematocyst knobs are contracted to such an extent in preserved medusae that they are difficult to observe.

TENTACLES

The tentacles in both living and preserved material were in poor condition. Most specimens have four large tentacles (as shown in Fig. 2) each associated with one of the four primary canals. Each of the other canals has a smaller tentacle which terminates in a nematocyst knob. The small, intermediate tentacles are usually not associated with canals, but in one specimen, erratic canal branches (Fig. 3 h) are sometimes associated with an intermediate tentacle.

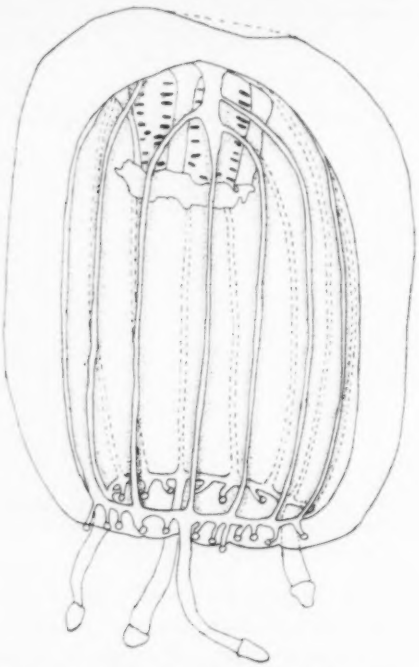

FIG. 2. A camera lucida drawing of *Calycopsis nematophora*, 28 mm high.

Bigelow (1913) believed that development of the tentacles precedes that of canals, and that each canal develops to associate with a tentacle. In the recent specimens some intermediate tentacles are not associated with canals.

The total number of tentacles ranges from 33 to 48. This agrees with the range given by Bigelow (1913).

CANALS

The genus *Calycopsis* is characterized by four primary radial canals and a varying number of centripetal canals which usually join at the base of the manubrium. Kramp (1959) has arranged the species of this genus from the simplest (with four primary canals and four centripetal canals which do not fuse with the base of the manubrium) to the most complex (with four primary canals and up to 56 centripetal canals which may or may not join with the manubrium). *Calycopsis nematophora* has four primary canals and 11–15 centripetal canals that join with the base of the manubrium (Fig. 2). It is, therefore, intermediate in complexity.

The most common canal pattern is shown in Fig. 3a. It was found in the three smallest, the largest, and four other medusae as well as in one or more quadrants of all other medusae. Usually there are three centripetal canals in a

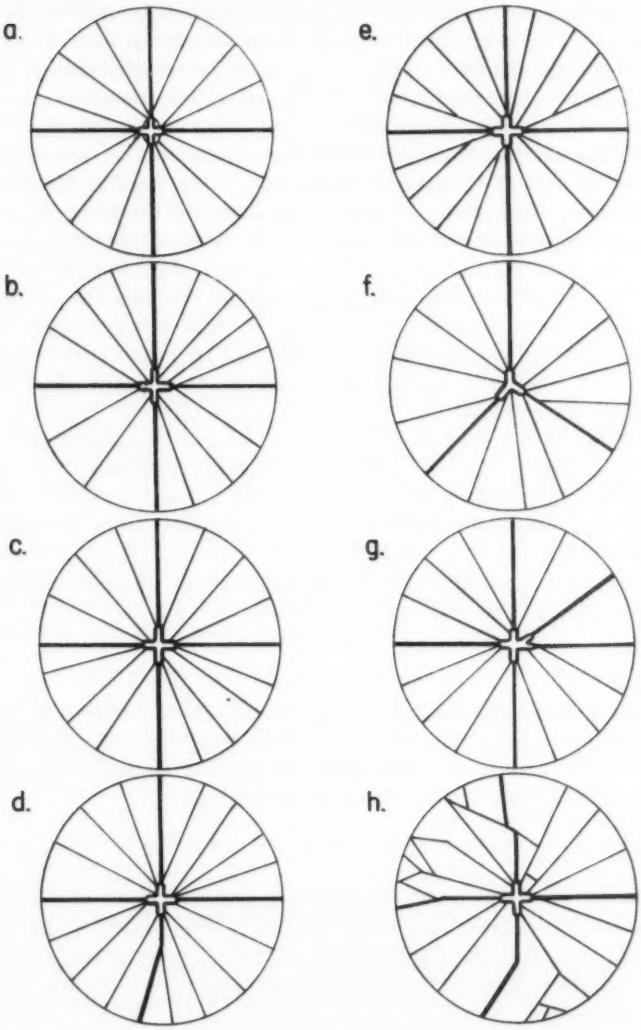

FIG. 3. Shematic drawing of the variation of raidal canals. a, common 16 canal pattern; b, variation of common pattern; c, 17 canals; d, 18 canals; e, 19 canals; f, three primary canals; g, five primary canals; h, branching centripetal canals.

quadrant which join with the cruciform base of the manubrium, although there is some variation. In four instances, a radial canal divided near the ring canal and rejoined near the manubrium. Two individuals had a transverse canal near the manubrium connecting two canals (Fig. 3h). The total number of canals varied from 14 to 19. The centripetal canals were complete except in one

specimen where three blind centripetal canals were present. Two of the three canals were in the same quadrant; the third was in another quadrant. The remaining two quadrants had three complete centripetal canals. The blind centripetal canals extended up from the ring canal a distance of 3, 4, and 6 mm. The different lengths suggest that these canals are not just sporadic outgrowths of the ring canal as Bigelow indicated with his specimen, but may be developing canals which eventually would have joined with the base of the manubrium. Variations of the common canal pattern are shown in Fig. 3b–e. In addition to showing variations in centripetal canal pattern, three specimens had anomalous primary canal structure (Fig. 3f and g), although they were normal in other respects. Two of the four specimens obtained in the samples off the Oregon coast have numerous branched canals (Fig. 3h) that recall features mentioned by Bigelow (1938) in two specimens of *Calycopsis* sp. taken near Bermuda. The aberrant canal condition of the Oregon specimens appears to be caused by extreme environmental factors.

The edges of the canals are smooth to ragged, the degree of roughness varying along any one canal or between canals. Bigelow (1913) associated roughness with the ability with which the canal can send out other canals, but in the present material roughness does not appear to be associated with additional canals.

Every canal, including the three centripetal canals, possesses a subumbrellar muscle band, a characteristic unique to *C. nematophora*.

Color

The color of the gonads, labial nematocyst knobs, and manubrium of the five live specimens was very dark red, almost purple, but after 30 days in preservative the manubrium and gonads were pale reddish brown, as Bigelow (1913) states, and the nematocyst knobs were white.

Nematocysts

The labial knobs and the intermediate tentacles were examined by the method of Mackie (Totton and Mackie, 1960, p. 394) to determine the type of nematocysts present. Desmonemes were found in both areas. Russell (1940) reported both desmonemes and microbasic euryteles for other members of the family Calycopsidae.

In 13 specimens of *C. nematophora*, several amphipods (*Parathemisto pacifica*) were found attached to the manubrium. Since no nematocysts were present in the stomach contents of five amphipods examined, there is no evidence that amphipods were feeding upon the medusae. Interspecific relationships are suggested but their nature is not known.

SUMMARY

Previously recorded only once from nine localities in the North Pacific, *C. nematophora* is reported from subarctic water in the Bering Sea, Gulf of

Alaska, and California Current, mainly from epipelagic waters. The three specimens from the California Current off Oregon differ from those collected further north in details of radial canals, possibly reflecting environmental differences.

A basic pattern of 16 canals, with three centripetal canals per quadrant, prevailed. Although considerable variation in this pattern was observed it was not related to umbrella height. The canals of the adult medusae are joined with the base of the manubrium, and every canal has a muscle band associated with it.

The only nematocysts found were desmonemes, and the amphipod *Parathemisto pacifica*, present on the manubrium, did not have any nematocysts in the gut.

ACKNOWLEDGMENTS

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NOTES

Myxosporidian Parasite from Salmon

Fish (1939) reported the presence of spores of *Henneguya salminicola* in the body musculature of coho salmon (*Oncorhynchus kisutch*), pink salmon (*O. gorbuscha*), and chinook salmon (*O. tshawytscha*). Spores, presumably of *Henneguya salminicola*, were found to be present in fresh and frozen samples of coho salmon (Patashnik and Groninger, 1964).

During an investigation of the storage of coho salmon in refrigerated sea water (−1°C) and refrigerated brine (−4°C), it was found when the fish were cut for canning that isolated areas of flesh extending lengthwise in the fish for as much as half their length were "milky." The "milky" effect was observed in three of eight fish stored 2 weeks, but not in fish from the same catch stored 2 days or 1 week (11 fish). It was extensive in two fish stored at −1°C, barely detectable in one stored at −4°C. These observations seem to indicate that the condition may appear or at least become more severe post-mortem.

Wet mounts and methylene blue stained smears prepared from "milky" flesh were examined under phase-contrast and bright-field illumination. Photomicrographs were taken of stained smears under oil immersion (magnification × 1125) and phase-contrast illumination. The photomicrograph presented in the accompanying Fig. 1 shows the type of spore found in the affected areas of salmon stored in either refrigerated sea water or refrigerated brine.

As reported by Patashnik and Groninger (1964) myxosporidian parasites causing the "milky" condition in certain species of fish may be readily identified by microscopic examination of affected tissue for characteristic spores; the present-day classification and identification of myxosporidia is based almost entirely upon the character of the spore. The characteristics of the spores

TABLE I. Comparative spore size of myxosporidia.

	Stained spores	Stained spores[a]	Unstained spores[b]
	μ	μ	μ
Length spore cavity	9–11	9.3	11
Breadth of spore cavity	7–9	7.3	7
Length — long tail	30–34	32.7	30
Length — short tail	25–27	25.5	20

[a]Reported by Fish (1939).
[b]Reported by Patashnik and Groninger (1964).

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observed microscopically in the present work were in agreement with the characteristics of spores of *Henneguya salminicola* which were obtained by Fish (1939) and Patashnik and Groninger (1964) from coho salmon. Each spore as described by Fish consisted of a spore cavity, two polar capsules, and two tails — one tail shorter than the other. Patashnik and Groninger referred to the spore cavity as an ellipsoidal body. Table I presents comparative data concerning the size of spores as obtained by the writers, by Fish (1939), and by Patashnik and Groninger (1964). It is concluded that the myxosporidian parasite obtained from coho salmon in this investigation closely resembles the parasite *Henneguya salminicola*.

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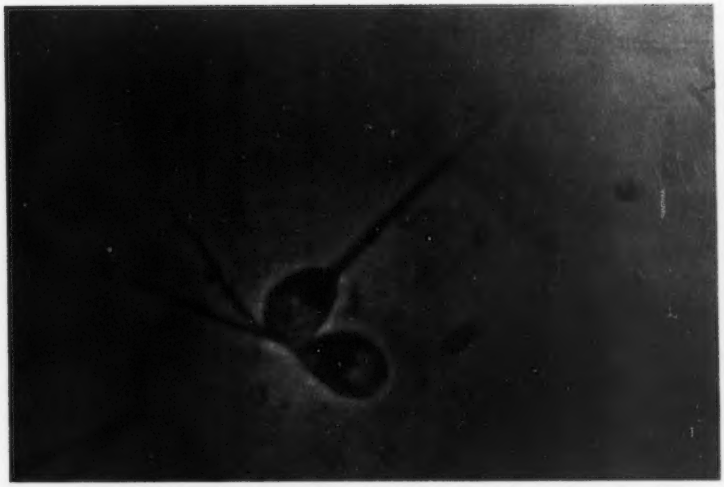

FIG. 1. Photomicrograph of spores of a myxosporidian parasite found in coho salmon. Methylene blue stained smear observed under oil immersion objective and phase-contrast illumination. × 1500.

Manganese Deposits on Shells of Eastern Mud Snails, *Nassarius obsoletus*, in Bideford River, Prince Edward Island

The eastern mud snail, *Nassarius obsoletus* (Say), is common up to high tide level in shallow, muddy locations in Prince Edward Island. The species is abundant in the Bideford River and it was noted that the shells of living specimens were encrusted with a thick black amorphous deposit. X-ray fluorescence analysis showed the layer to be composed principally of manganese and iron in the ratio of 5:1.

Deposition of manganese on the shells of living and dead molluscs is a widely known phenomenon, at least from sub-tidal species (Allen, 1960). Manganese is apparently deposited by both biological and inorganic processes but always at the immediate interface of sea and substrate. Allen considered that deposits on living molluscs were the result of biological activity rather than the adsorption of precipitated particles.

The examination of over 1000 shells of *N. obsoletus*, both living and dead, from Bideford River showed that manganese deposits were present only on living individuals. Fragmentary deposits were sometimes present on recently dead specimens but long-dead individuals showed no manganous layer. The thickness of the layer was proportional to the size of the shell; the thickest layer measured 1.1 mm on an individual 27 mm in length, about the maximum size for this species. The layer was present over the entire outer surface of the shell, but not on the operculum. Near the lip and on small shells the deposit was smooth but toward the apex of older specimens the layer was easily dislodged, and consequently often uneven.

The deposit probably has a biological origin because it forms only on living specimens. It appears to become detached from the shell soon after the death of the snail. It is unlikely that the layer is mechanically eroded from dead shells since the substratum is very soft and water currents slight. Further evidence of a biological origin is the specificity of the layer to *N. obsoletus*. Manganese deposits of this nature have not been observed on any other mollusc in the same general habitat, curiously not even on *N. trivittatus*, a closely related species with a similar and, if anything, rougher shell texture.

Many investigators have remarked on layers of mud and algae on *N. obsoletus* shells (Abbott, 1954; Bousfield, 1960; Smith, 1937). Analysis of such layers from other areas would be worthwhile to check whether a manganous deposit is typical of this species.

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I am indebted to Mr G. Lachance, Geological Survey of Canada, Ottawa, who carried out the X-ray fluorescence analysis, and to Dr A. H. Clarke, Jr., National Museum of Canada, Ottawa, for advice.

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Sarda lineolata (Girard), a Pacific Bonito, in the Strait of Georgia, British Columbia

On June 19, 1964, about 0400 hr, while fishing commercially with a surface gillnet for coho salmon (*Oncorhynchus kisutch*) about "2 or 3 miles north of Yellow Point" (49°02′N lat, 123°45′W long) on the east coast of Vancouver Island, British Columbia, Mr J. Fraser caught a Pacific bonito (*Sarda lineolata*), frequently called California bonito. The fish (Fig. 1) was examined and identified using keys published by Walford (1937) and Godsil (1955).

External body measurements and meristic counts made on the specimen are listed in Table I. All measurements were made as closely as possible according to methods used by Godsil and Byers (1944) after the specimen had been thawed from its frozen state. Certain body proportions differed from those for other specimens examined and listed by Godsil (1955), but these differences may be the result of comparing measurements from frozen and fresh individuals. Regarding countable characters, the Strait of Georgia specimen has one more gill raker on the lower limb of the gill arch.

In addition to morphological differences, the specimen also possessed some external features which apparently were lacking or went unnoticed in other specimens examined. The lateral line system clearly consisted of 1 main line and 2 distinct secondary lines, 1 above and 1 below the anterior end of the main lateral line. Also, the main line possessed a short branch line in the area below the second dorsal fin. The position and external course traversed by the lateral line system are indicated in the outline diagram in Fig. 1.

Characteristically, the black stripes, 7 in number, were nearly horizontal posteriorly to about the area below the second dorsal fin, and then ran obliquely upward. On the right side, however, the fourth lowest stripe divided into 2 branches anteriorly from a position below the third last spine of the first dorsal fin, indicating that the markings on each side were not the same.

The fish, measuring 618 mm in length and weighing 2795 g, was a maturing female possessing large ovaries with readily recognizable ova.

Pacific bonito normally range from southern California to the northern end of Vancouver Island (Clemens and Wilby, 1961) and are most abundant between Santa Barbara and Magdalena Bay (Walford, 1937). In warm anomalous years they may be numerous as far north as northern California (Radovich, 1961), and occur northward to coastal waters of central Alaska (Quast, 1964). In British Columbia single specimens have been caught previously in Rivers Inlet and near Sooke in Juan de Fuca Strait (Clemens and Wilby, 1961). The present specimen not only represents the third record for British Columbia but is the first bonito from the Strait of Georgia.

Sea surface temperatures observed at monitoring stations at the approaches to and in the Strait of Georgia indicate that water temperatures in October 1963 were significantly warmer than the 10-year mean for that month, and by April 1964 were possibly colder than average (Hollister, 1964). While the mean October temperature differed to some extent between stations, in general

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TABLE I. Comparison of body measurements and counts made on the Strait of Georgia specimen of *Sarda lineolata* with ratio measurements for *S. lineolata* recorded by Godsil (1955). Eye and maxillary measurements are related to head length; all other measurements are related to body length.

Anatomical character	Strait of Georgia specimen		*S. lineolata* (8) range in ratios
	Measurement (*mm*) or count	Ratio	
Body length	618		439–656
Head length	147	4.20	3.70–4.02
Snout to insertion first dorsal	155	3.99	3.46–3.85
Snout to insertion second dorsal	349	1.77	1.71–1.80
Snout to insertion anal	396	1.56	1.51–1.58
Snout to insertion ventral	166	3.72	3.33–3.57
Greatest body depth	129	4.79	4.35–4.99
Greatest body width	86	7.19	
Ventral insertion to vent	230	2.69	2.57–2.91
Length 1st dorsal base	176	3.51	3.30–3.44
Length 2nd dorsal base	54	11.44	10.18–11.86
Length of anal base	44	14.05	13.05–16.13
Pectoral length	77	8.03	7.32–8.28
Height of 1st dorsal	ca. 58	10.66	8.62–10.97
Height of 2nd dorsal	49	12.61	11.31–12.54
Height of anal	42	14.71	12.15–14.16
Diameter of iris	16	9.19	9.33–10.19
Maxillary length	70	2.10	2.05–2.15
Snout to posterior margin of eye	66	2.23	2.21–2.28
1st dorsal rays	18		18–19
2nd dorsal rays	13		14–16
Dorsal finlets	8		7–9
Anal rays	13		12–13
Anal finlets	7		6–7
Gill rakers			
Upper limb	8		7–10
Angle	1		1
Lower limb	19		11–18
Total count	28		20–27

these approximated the lower limit of the temperature range of distribution (12°C) which Laevastu and Rosa (1962) quote for bonito. It is reasonable to think therefore that the individual reported here moved into the Strait of Georgia during the period of abnormally high water temperatures and remained in coastal seas during the winter, rather than entered these waters in the spring of 1964. The capture of bonitos in late November 1963 in lower Puget Sound (Mr D. Kauffman, Washington Department of Fisheries, personal communication) supports this view.

The bonito reported here has been deposited at the Institute of Fisheries, University of British Columbia, Vancouver, B. C., and has been assigned catalogue number BC 64-394.

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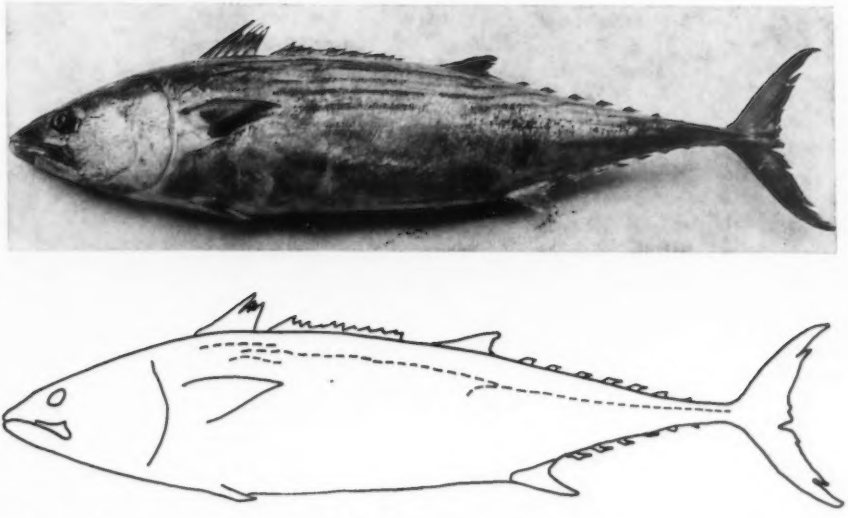

FIG. 1. Photograph of Pacific bonito (*Sarda lineolata*) caught in the Strait of Georgia, and an outline diagram showing the lateral line system (dotted lines).

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A Juvenile Six-Gilled Shark (*Hexanchus corinus*) from the San Juan Islands, Washington[1]

A small (T. L. 750 mm) male, six-gilled shark, *Hexanchus corinus* (Jordan and Gilbert) 1880 (Fig. 1) was caught on October 23, 1964, during otter trawling operations in 15 fathoms of water in East Sound, Orcas Island. Bottom temperature at time of capture was 9.5°C. Such captures are rare, the only comparable specimen we know of from the Pacific coast being a 600-mm fish from California (Regan, 1905). Our specimen has been referred to *H. corinus*, rather than *H. griseus* (Bonnaterre), since the previous attempts to synonymize these two species have been based on insufficient information (Mr Stewart Springer, personal communication).

Upon capture, the shark was placed in a live tank on board the M. V. *Hydah* (Friday Harbor Laboratory vessel) and then later transferred to an annular tank at the marine laboratory. The shark had many superficial scratches (which were apparently received while in the trawl) but otherwise appeared healthy. During a period of 1 week in this tank the shark was very lively, but was observed to be unable to avoid contact with the side of the tank as it swam continuously around it. This resulted in blindness in the right eye. The shark was transferred to the larger annular tank at the College of Fisheries, University of Washington; during this handling it made repeated attempts to bite its captors, a trait not usually associated with the larger adults. In spite of the greatly increased tank area to swim in, the shark continued to make contact with the sides and soon had scraped part of its snout away and chaffed its left eye. Again the fish was transferred, this time to a rectangular, fiberglassed tank which had a much smoother surface than the annular tanks. However, on November 6 the shark was observed to be dying and was immediately frozen. Four days later it was thawed and photographs and measurements were taken. At this time an umbilical scar, 46 mm in length, was observed to be present at the midline, approximately between the pectoral fins.

Never during its captivity did the shark feed although several kinds of its reported food items (Clemens and Wilby, 1961; Bigelow and Schroeder, 1948) were tried, using methods which had previously been successful with dogfish sharks at the College of Fisheries. Force-feeding, however, was not attempted.

With the recent capture of the juvenile *Hexanchus*, an excellent opportunity was presented to compare the teeth from the lower jaw of the juvenile with those of two adult sharks (one male, T. L. 7 ft, 5¾ inches, and one female, T. L. 9 ft, 2½ inches), which were recently captured (Spring, 1964) in Elliot Bay, Puget Sound, by the staff of the Seattle Aquarium. These specimens were deposited in the College of Fisheries. Bigelow and Schroeder (1948) state that there is an increase in the number of cusps on the teeth with growth.

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[1]Contribution No. 189 from the College of Fisheries, University of Washington.

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In the two adult jaws examined, the number of cusps ranged from 8 to 10, with the inner margins of the teeth finely serrate. The teeth of the juvenile lacked the finely serrate inner edges and possessed 5–6 cusps. The first 2 anterior trapezoid teeth on the side of the lower jaw possessed 5 cusps, while the more posterior teeth increased to 6 cusps. These counts confirm Bigelow and Schroeder's statement.

A complete set of measurements of the juvenile specimen is presented below, since the morphometry for a Pacific coast six-gilled shark of this size has not been previously recorded in the literature. All measurements are the straight line distance between two points, in millimeters, and are usually self-explanatory. A guide to the terminology used in the measurements may be found in figure 1 of Rosenblatt and Baldwin (1958).

Total length: 750 mm.

Round weight: 1085 g.

Snout tip to: outer nostrils 8.8; eye 29; spiracle 82; mouth 33; 1st gill opening 112; 2nd gill opening 118; 3rd gill opening 124; 4th gill opening 128; 5th gill opening 133; 6th gill opening 136; pectoral insertion 138; pelvic insertion 307; cloaca 343; dorsal origin 375; anal fin origin 405; upper caudal origin 484; lower caudal origin 488 mm.

Distance between bases of fins: dorsal and caudal 64.8; pectoral and pelvic 126; pelvic and anal 51.6; anal and caudal 41.2 mm.

Distance between insertions of pectoral and pelvic: 181.9 mm.

Distance between inner corners of nostrils: 32 mm.

Mouth: width 59.2; length 26.3 mm.

Gill opening length: 1st 45.4; 2nd 40.5; 3rd 42.5; 4th 40.1; 5th 35.8; 6th 30.0 mm.

Eye: horizontal diameter 22.1; vertical diameter 13.5 mm.

Dorsal fin: overall length 60.0; length base 42.7; length posterior margin 15.5; height 27.5 mm.

Anal fin: overall length 49.5; length base 36.7; length posterior margin 12.0; height 22.0 mm.

Pectoral fin: length base 49.9; length anterior margin 97.0; length distal margin 53.5; length posterior margin 35.0 mm.

Pelvic fin: overall length 71.2; length base 53.2; length anterior margin 42.5; length distal margin 46.0 mm.

Caudal: length dorsal lobe 263; ventral lobe with tip 180 mm.

Trunk at pectoral origin: width 89; height 74.2; girth 225 mm.

The specimen has been deposited in the College of Fisheries Museum, University of Washington, No. 18500.

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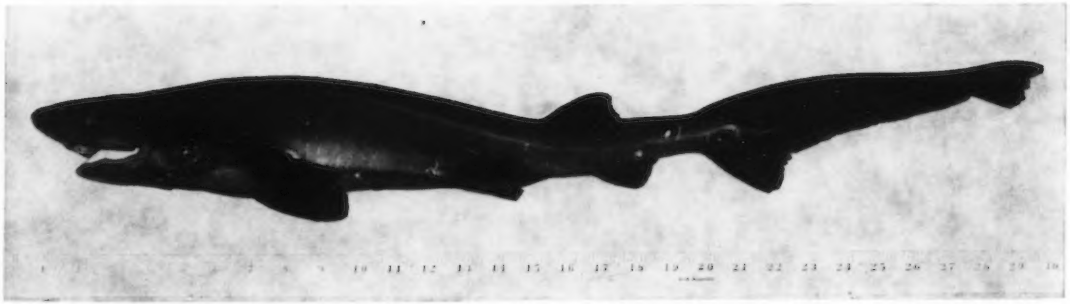

FIG. 1. Photograph of a juvenile *Hexanchus corinus* from the San Juan Islands (rule is in inches).

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Orange-Red Meats in Sea Scallops

Orange-red-coloured flesh in groundfish is rare but it has been described by several authors. Bligh and Dyer (1959) reported orange-red flesh in cod and haddock and found that astaxanthin and zeaxanthin, present in a 2:1 ratio, were responsible for the colour in cod flesh. In haddock, the colour was caused entirely by zeaxanthin. Recently, Rae and Johnson (1963) reported orange-red-coloured flesh in haddock and dab. They attributed the colour to minute traces of astaxanthin and taraxanthin. In these species, these compounds are normally localized in the skin and not in the flesh.

Although the typical colour of the adductor muscle ("meat") of sea scallops (*Placopecten magellanicus* Gmelin) is white, orange-red-coloured meats are also found. Fishermen call these scallops "salmon scallops." In contrast to pigmented groundfish flesh, salmon scallops are quite common and are found throughout most of the range of sea scallops in the northwest Atlantic. Orange-red meats have been reported from Georges Bank, the Bay of Fundy, the Gulf of St. Lawrence, St. Pierre Bank, and around parts of Newfoundland. Scallops with coloured meats appear to be as healthy as animals with normally coloured white meats.

In this brief study an assessment was made of the intensity of pigmentation in scallop meats, their distribution and abundance, and the compounds responsible for the colouring. Most of the observations were made on scallops from the major fishing area, Georges Bank, but scallops from the Bay of Fundy and the Gulf of St. Lawrence were also examined.

The intensity of the colour in pigmented scallop muscles varied from barely coloured meats to those which were a deep orange-red; the colour was evenly distributed through the tissue. (Colour photographs are available at the St. Andrews Biological Station showing this gradation in colour.) In a few animals the meats were white but had five to ten 2-mm fibers of orange-red colour going down through the muscle. Scallops with deeply pigmented meats also had the orange-red pigmentation distributed through other organs, particularly the gills and mantle. The orange-red pigmentation may also be dispersed through gill and mantle tissue in animals with less deeply-coloured meats but it was not noticeable to the naked eye.

The sexes of sea scallops are separate: the male gonad is milky white; the female gonad is pink when it begins to fill out but turns a bright coral-red when ripe. Over 5000 scallops with orange-red meats were examined and they were always females. Not all meats in female scallops are coloured and, as noted previously, there was a great variation in intensity of colour but

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the pigmentation in meats apparently is associated with the colour in the female gonad.

On Georges Bank, scallop gonads begin to fill out in early March and spawning occurs in late September or early October. Orange-red meats were found in Georges Bank scallops throughout the sampling period, May to October. Although deeply-coloured meats were seen in May, visual observations indicate that the intensity of colour increased slightly as the season progressed.

Because of their uneven distribution, it was not possible in this brief study to determine if the percentage of coloured meats in a population increased from May to October. No winter observations were made from Georges Bank but fishing skippers have reported that coloured meats are much rarer in winter than in summer. Winter observations on Bay of Fundy scallops showed that coloured meats were rare at this time of year, but a few coloured meats were seen in October which is about 1 month after the animals spawn. The colour in these meats was not particularly intense.

Larger scallops had a higher proportion of orange-red meats than smaller scallops. In a sample of 1000 animals taken from the northern edge of Georges Bank on July 3, 1960, 24% of the scallops over 100 mm shell height had coloured meats whereas only 11% of meats from animals under 100 mm were coloured. In another sample of 500 animals taken from the same area on August 1, 1962, coloured meats were found in 24% of the larger scallops but in only 13% of the smaller ones. Sea scallops probably first spawn as 3-year-olds; the gonads of 65-mm scallops (shell height) are well developed and the sexes can be easily distinguished.

Although orange-red meats were found in the Bay of Fundy and frequently in the Gulf of St. Lawrence, they were more common on Georges Bank and occurred widely over this bank. On Georges Bank pigmented scallops were found on the four major fishing areas — northern edge, northeast peak, southeast part, and the channel. In any particular area the distribution of coloured meats was patchy but they were most common on the northern edge of the bank and least abundant on the southeast part. However, even on the northern edge their distribution was patchy (see Table I) and often a shift of a few miles changed the percentage of coloured meats in the catch from 30% to less than 5%.

Chemical analyses were carried out on samples of gonads and deeply-coloured meats to identify the pigment and to determine if it was the same in both meats and gonads. The samples were collected from the northern edge of Georges Bank on July 5, 1960, and the methods used in the analyses were the same as previously described by Bligh and Dyer (1959). From the analyses it was concluded that the pigmentation of the adductor muscle was due to the presence of zeaxanthin. This sample of deeply-pigmented meats contained about 0.2 mg zeaxanthin per 100 g tissue. In a previous investigation (Bligh

TABLE I. Percentage of orange-red meats in scallops from different areas of the northern edge of Georges Bank which had a high proportion of coloured meats.

Date	Total number of meats counted	Number of orange-red meats	Percentage of orange-red meats in total
(Oct. 11, 1959 — 25% of meats from females were orange-red)			
June 29, 1960	200	49	24.5
July 3, 1960	1000	255	25.5
July 5, 1960	200	39	19.5
May 24, 1961	211	9	4.3
May 29, 1961	127	39	30.7
May 31, 1961	58	14	24.1
June 2, 1961	117	36	30.7
July 30, 1962	200	23	11.5
Aug. 1, 1962	500	96	19.2

and Dyer, 1959), this same carotenoid was found in a pigmented haddock, and again no other carotenoid pigments were detected in appreciable concentration.

Zeaxanthin was also identified as the major pigment of the intensely-coloured gonad tissue. The chromatographic analyses indicated that other carotenoid pigments were present in this tissue but they were not identified. On the basis of ultraviolet absorption analyses, the zeaxanthin concentration (per unit weight) in the gonad was about 40 times the concentration in the adductor muscle.

It is hard to explain the deposition of this pigment in the adductor muscle, and in some other tissues, and why it occurs in some scallops and not in others. However, since pigmented meats were only found in female scallops where pigment deposition in the gonads is a natural phenomenon, it would appear that this is merely a side effect of gonad maturation and development. The variation in diet among scallops on the different fishing grounds may be an important factor, or some females may secrete a super abundance of zeaxanthin whereas others may be better able to eliminate it.

Coloured scallop meats have caused a minor problem in industry since many people refuse to eat them believing they are spoiled white meats. Some plants even culled the deepest coloured meats from the catch. Apart from the pigmentation, the water, fat, and protein content appeared to be the same for both coloured and noncoloured meats. Also, no difference in taste was

detected or has been reported. Thus, there is no reason why orange-red scallop meats should not be just as wholesome as white meats.

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Candling for the Detection of *Triaenophorus crassus* Cysts in Whitefish

Whitefish (*Coregonus clupeaformis*) from some Canadian lakes frequently are infested with cysts of the tapeworm *Triaenophorus crassus* (Lawler, 1959). Although these cysts are harmless for human consumption, infested whitefish are curtailed in trade. In order to maintain the considerable export of whitefish to the United States, detection and removal of the cysts is required. At present, this is done by candling, that is the transillumination of the skinless fillets, and manually cutting out any cysts that are detected. This process, however, is not fully effective and closer inspection sometimes reveals cysts after the fillets have passed through an industrial candling process.

In an endeavour to improve existing candling methods the optical problems in industrial candling were investigated.

Previous investigations (McMurtrie and Carter, 1948; Power, 1958) showed that larger cysts or parasites in fillets were detected only if they were located in an upper layer of flesh less than $\frac{1}{2}$ inch thick. Colored or polarized light did not improve the efficiency of candling. Scattering of light was concluded to be the major limiting factor.

Based on this result some thought was given to the optical instrumentation used in the candling process. In most of the previous investigations the candling apparatus produced diffuse light for the transillumination of the fillets. However, it must be expected that an opaque object imbedded in the middle of a scattering material will produce a stronger shadow at the upper surface of the material if transilluminated with collimated (unidirectional) light rather than with diffuse light. Moreover, an even darker shadow should occur if light enters the scattering medium only at a small area beneath the opaque object and not at an area many times larger than the object.

To verify these theoretical considerations a model of the whitefish, with an imbedded cyst, was made by sandwiching a strip of paper (3 mm wide, 50 mm long) between two pieces of pot-opal glass (each 50 × 50 × 2 mm) that served as the scattering layer. In transillumination, the position of the paper strip could be seen on the surface of the opal glass as a narrow shadow with blurred edges. The contrast of this shadow against the background was measured photoelectrically under the following conditions and with the following results:

1. Diffuse light entering at the total bottom surface of the model.
   Contrast: 10.0%
2. Collimated light entering at the total bottom surface of the model.
   Contrast: 13.9%
3. Collimated light entering the model through a 3-mm-wide slit.
   Contrast: 26.6%

Thus the contrast of the shadowed area against the unshadowed area was more than doubled using a narrow band of collimated light for the transillumination of the model rather than a large area of diffuse light.

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Candling of skinless fillets was then studied at an industrial fish processing plant. Here, the candling table had four places for candling mounted in a flat roof-shaped table top, two places on either side of the ridge. Each position consisted of a clear glass plate, level with the stainless steel top of the table, and a 100-w incandescent lamp with frosted bulb mounted below the glass plate.[1]

On this candling table cysts near the upper surface of the fillet are spotted immediately and removed. To detect cysts in the thick part of the fillet near the backbone where cysts occur most frequently, several deep cuts, made parallel to the length of the fillet, were pried open, and inspected.

To apply the conclusions drawn from the experiments, one glass plate was covered with black paper except for a narrow slit located above the lamp bulb. Skinless fillets were candled on the candling position thus modified, moving the fillet slowly across the open, illuminated slit, and then over an unmodified position. However, modification of the candling table did not result in a significant improvement in the detectability of cysts.

The visual appearance of the fillets on the modified candling table demonstrated clearly that heavy scattering of light occurs in the fillets. Only through the thinnest part of the fillet could the slit-shaped aperture be recognized. If the thickness exceeded approximately $\frac{1}{4}$ inch the narrow band of light spread out into a bright area many times the width of the slit with a gradual decrease in brightness towards the ends of the fillet. This scattering allows the light to bypass any small obstacles which are not located near the upper surface of the fillet and thus limits the detectability of such obstacles.

It was concluded that the efficiency of visual candling of whitefish cannot be improved significantly beyond its present state due to the scattering of light of the visible spectrum which limits the detectability of cysts.

One additional remark may be made: The heat produced by the incandescent lamps of the candling tables may be detrimental to the fillets. Use of fluorescent lamps of a blue shade would not only keep the temperature of the glass plate down but might also improve slightly the contrast of the yellowish cysts against the white flesh.

This investigation was carried out at the request of the Fisheries Research Board of Canada.

It is a pleasure to thank Mr G. L. Grant, Area Director of Fisheries, Winnipeg, and his staff for the many fruitful discussions and assistance given in conducting the experiments at the fish processing plant.

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[1]A good illustration of a candling table may be found in National Geographic Magazine, Vol. 120, p. 778 (1961).

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Haematological Study of the Hake (*Merluccius merluccius*) from the Southwest Atlantic

Little information appears to be available in the literature on the haematology of fishes from the southwest Atlantic. During a series of studies on the bacteriology of commercially important marine fishes from Argentina, the authors were able to realize a parallel investigation on the haematology of such species, and the present communication presents results obtained from the hake (*Merluccius merluccius hubbsi*).

A total of 25 hake was used for this work, the fish being caught in the vicinity of Mar del Plata during the period June–September 1964. On their arrival in port, the fish were immediately transferred to the laboratory and following the taking of skin swabs for bacteriological studies, the fish were opened so as to expose the internal organs *in situ*. In each case the blood sample was obtained by cardiac puncture, using a heparinized hypodermic syringe, and the specimen was maintained subsequently in a small heparinized glass container. The haematological studies were carried out as follows:

Red cell count. The blood was diluted 1:200 in a Thoma red cell pipette, using Young's marine teleost saline (Hale, 1958) as diluent.The count itself was performed in an improved Neubauer haemocytometer.

Haemoglobin estimation. The estimation of the haemoglobin content was performed by the acid haematin method of Sahli (Kolmer and Boerner, 1944). This method nevertheless has the disadvantage that abnormally high readings tend to be obtained with fish blood owing to the presence of nucleated erythrocytes, which increase the turbidity of the resulting test fluid. To avoid insofar as was possible any errors due to this artificial increase in turbidity, the readings obtained with the Sahli haemoglobinometer were corrected by reference to the factor proposed by Larsen and Snieszko (1961) for fish blood, which can be expressed as:

$$0.534 \times \text{AH Hb value} + 2.44$$

where the AH Hb value corresponds to the acid haematin haemoglobin reading of the test blood.

Microhaematocrit. The heparinized blood was drawn up in a capillary tube, one end of which was subsequently sealed with a plasticine plug. The tube was then centrifuged for 30 min at 3000 rpm, followed by a further 5 min at the same velocity to ensure that the reading obtained was constant. The packed cell volume was expressed as a percentage by reference to an arbitrary scale prepared in the laboratory.

Absolute values. The mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), mean corpuscular volume (MCV), and colour

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TABLE I. Haematological study of the hake (*Merluccius merluccius hubbsi*) from the southwest Atlantic.

	Minimum	Maximum	Average
Red cell count (millions/cm³)	1.42	2.24	1.80
Haemoglobin (%)	39	48	43
Haemoglobin (g/100 ml)	5.8	7.2	6.4
Microhaematocrit (%)	31	42	36
Mean cell haemoglobin (μg)	30	44	36
Mean cell haemoglobin concentration (%)	–	–	17.5
Mean corpuscular volume (μ^3)	–	–	200
Colour index	1.01	1.34	1.19

index (CI) were calculated in accordance with standard procedures, assuming in every case that 100% haemoglobin = 14.8 g/100 ml.

The results obtained are given in Table I. The maximum and minimum figures obtained for the red cell count, haemoglobin concentration, and microhaematocrit, respectively, are given, whilst the average of the 25 readings obtained appears as the tentative normal for the species.

The methods employed during this study were found to be adequately adapted to fish blood, and no difficulties were experienced with the use of heparin. It is also of interest to note that no cellular deformation or crenation was observed in the red cells where these were diluted with Young's marine teleost saline.

In practice it was found that a very insignificant variation was present between the individual readings and the tentative normal calculated from the average of all results obtained. The authors are thus confident that the results presented in this communication may prove to approximate closely the true figure for this species of fish. Future work will therefore be designed to determine whether a similar picture may be obtained with other species of marine teleosts from this area.

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